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THE EFFECTS OF METHOXYCHLOR ON AQUATIC BIOTA

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ABSTRACT

Methoxychlor inhibited phytoplankton production at a concentration of 50 μg/l, however the effect may have been overestimated due to inhibitory effects of ethanol used as a solvent.

Rate of breakdown of methoxychlor in water varied considerably with the biological activity in the water. Methoxychlor was rapidly incorporated in the particulate fraction of the water by adsorption or metabolism.

Continuous-flow bioassays yielded 96-hour TL_{50} values for invertebrates ranging from 0.61 μg/l for Gammarus pseudolimnaeus to 7.05 μg/l for Orconectes virilis. Fathead minnows (Pimephales promelas) and yellow perch (Perca flavescens) had 96-hour TL_{50} values of 8.63 and 22.2 μg/l respectively.

Hatching of fathead minnow eggs was inhibited at all levels of exposure tested between 1.0 and 0.125 μg/l. There was no spawning at 2 μg/l.

Growth of yellow perch was retarded at all levels tested between 5.0 and 0.625 μg/l. All perch died at 10 μg/l during the growth study.

Perch which had been subjected to long-term exposure to 5 μg/l of methoxychlor had an abnormally high oxygen demand when held in a respirometer with a water velocity of 0.6 foot per second.

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SECTION I

CONCLUSIONS

Methoxychlor is highly toxic to aquatic organisms and continuous exposure to concentrations less than 1.0 μg/l can cause serious sublethal effects. Breakdown is rapid in productive waters, but could be slow enough to result in extended exposure periods in very unproductive waters. Gammarus, an important fish food organism, would probably be the most seriously damaged organism in an aquatic system. Continuous exposure of fish to sublethal levels will reduce growth, hatching success of eggs, and the ability to withstand stress.

SECTION II

RECOMMENDATIONS

The study of sublethal effects of methoxychlor should be extended to include field studies in natural habitats. This is being started now in the artificial streams constructed as part of this project.

In the future, we should watch for the accumulation of significant background levels in areas where insect control programs require frequent repeated applications of methoxychlor. Levels sufficient to cause sublethal effects are especially apt to persist in unproductive waters.

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SECTION III

INTRODUCTION

The purpose of this study was to determine effects of methoxychlor on the aquatic system with emphasis on the sublethal effects of long-term exposure on fish and invertebrates.

Numerous studies in the last several years (Burdick et al., 1964; Peakall, 1970; Reinert, 1969; and Wurster, 1968) have shown a variety of environmental problems attributable to the widespread use of DDT. Awareness of these studies, by an aroused public, has resulted in the complete banning of DDT. Methoxychlor was one compound recommended to replace DDT in many control programs especially those for the European bark beetle and black fly larvae. Methoxychlor was recommended because it is considered to be persistent but biodegradable, and readily metabolized when ingested by living organisms. Consequently it would not concentrate in the food chain.

Current literature agrees that the rate of breakdown of methoxychlor is rapid in natural waters. Burdick et al. (1968) found that ponds treated with 5 μg/l of methoxychlor contained undetectable concentrations when sampled 36 days later. Wallner et al. (1969) concluded that a helicopter application for European elm bark beetles was probably not hazardous to aquatic life since fallout on a stream was quickly diluted and moved downstream with little storage in the bottom sediments. Approximately 1 μg/kg of methoxychlor persisted in the bottom silt from spring through fall.

Rheinbold et al. (1971), Kennedy et al. (1970), and Metcalf et al. (1971) all found methoxychlor to be much less concentrated than DDT in tissues of fishes and invertebrates. The only noted exception was snails which concentrated twice as much methoxychlor as DDT (Rheinbold et al., 1971). However, Kruzynski and Leduc (1972) documented fish mortality and tissue concentrations as high as 2.65 mg/kg from spray applications of as little as 0.2 pound per acre methoxychlor. They also found in their feeding experiments (0.01 to 2.0 mg/kg/day) that brook trout retained about 80% of the ingested insecticide resulting in concentrations up to 45 mg/kg in whole fish tissue. This concentration caused anemia and severe tissue damage in the liver and kidney. These authors indicated tissue damage was synonymous with concentration, however I do not believe anyone has successfully demonstrated that concentration necessarily precedes sublethal effects.

All studies to date have consisted of low-level feeding experiments or short-duration exposure studies. Most exposure studies have been

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based on a single application which, because of rapid breakdown, results in very short exposure time. Kennedy et al. (1970) conducted a chronic study with bluegills in ponds, however it was a single-treatment method, and he did not monitor methoxychlor concentrations.

Despite the rapid breakdown rate of methoxychlor, any use that demands frequent repeated applications (such as fruit orchards) will result in maintaining significant background levels in our natural waters. For this reason we instigated the present study to determine the effects of long-term exposure of aquatic organisms to sublethal levels. The study has consisted of the following segments:

1. Effects of methoxychlor in phytoplankton production in Third Sister Lake, Washtenaw County, Michigan, as measured in experiments with C^{14} in bottles.

2. Rate of breakdown of methoxychlor in waters varying in pH and hardness.

3. Acute toxicity of methoxychlor to fathead minnows and yellow perch in both static- and continuous-flow bioassays.

4. Chronic toxicity to fatheads and perch as interpreted by effects on growth, hatching success of fathead eggs and respiration requirements of perch.

5. Acute and chronic continuous-flow bioassays conducted on various stream- and pond-dwelling aquatic invertebrates. Acute bioassays were run for 96 hours on Stenonema interpunctatum (group) (mayfly), Chironomus tentans (midge), Cheumatopsyche sp. (caddisfly), Gammarus pseudolimnaeus (scud) and Orconectes virilis (crayfish). Chronic bioassays were run for 28 days on Stenonema terminatum (group), S. interpunctatum (group), and C. tentans. Chronic bioassays were run for 42 days for Cheumatopsyche sp. and G. pseudolimnaeus.

SECTION IV

METHODS

Primary production. -- The procedure for determining the effect of methoxychlor on primary production consisted of dosing 5-gallon Pyrex jugs of lake water from Third Sister Lake with different combinations and concentrations of alcohol and Triton X-100 solutions of methoxychlor, and incubating the jugs in the lake at a depth of 1 meter. The jugs were then periodically sampled for productivity determinations and methoxychlor analysis.

Water was pumped from the lake at a depth of 1 meter into a 150-gallon capacity polyethylene drum. The jugs, previously acid-cleaned and rinsed with distilled water, were filled randomly in three "slugs" by gravity flow from the drum. The water in the drum was continuously mixed with a plastic plunger during the jug-filling period. Upon completion of the filling procedure the jugs were covered with black polyethylene bags to minimize light shock to the algae in the water.

The jugs were treated with methoxychlor in alcohol and Triton X-100 solution. One milliliter of solution was used in all cases. The treatment was as follows:

a. Control--no additive.

b. ETOH--1 ml of 95% ethanol.

c. Triton X-100--1 ml of a solution composed of 8 drops of Triton X-100 in 100 ml of distilled water.

d. ETOH/Triton X-100--1 ml of a solution of 95% ethanol plus 1 ml of the Triton noted above.

e. 1 μg/l methoxychlor--1 ml of a solution containing 0.02 mg technical grade methoxychlor, in 95% ethanol with 8 drops of Triton X-100 per 100 ml of solution.

f. 10 μg/l methoxychlor--1 ml of a solution containing 0.2 mg technical grade methoxychlor in 95% ethanol with 8 drops of Triton X-100 per 100 ml of solution.

g. 50 μg/l methoxychlor--1 ml of a solution containing 1 mg of technical grade methoxychlor in 95% ethanol with 8 drops of Triton X-100 per 100 ml of solution.

Each of the above treatments was done in duplicate. The lake was also sampled along with the treatments using a Van Dorn bottle to show the effect of containment.

The jugs were suspended and incubated in the lake at a depth of 1 m. This was accomplished by suspending the jugs from a series of floats anchored in the middle of the lake in the form of a square approximately 50 feet on a side. The jugs were secured to the floats by means of snap clips and nylon lines affixed to the jug harness. The jugs were, in this manner, incubated at a depth of 1 m for the entire period of the experiment.

The jugs for the first run, 8-13 July, were dosed with the required solutions after being suspended in the lake. This was accomplished by volumetrically pipetting the above mentioned solutions into the jugs while mixing with a plastic plunger. The jugs for the second run, 3-13 August, were dosed prior to suspending them in the lake.

Finally, the jugs were sampled by the use of a vacuum pump arranged to evacuate the sample bottles by pulling the water from the jugs into the sample containers. The volume withdrawn for the various analyses was as follows:

a. Productivity--125-ml aliquot

b. Methoxychlor--two, 250-ml aliquots

To determine the productivity, the jugs were sampled in the manner described above. Three 125-ml Pyrex bottles were filled from the jug. One of the bottles was covered with aluminum foil. Each bottle was then injected with 1 ml of a 0.5-mc solution of $NaH^{14}CO_3$. A scintillation standard was prepared for each run. The bottles were then placed horizontally on a rack and suspended at a depth of 1 m in the lake.

For the first run, the dark bottles (aluminum-foil covered) were incubated on the rack with the light bottles. For the second run, the dark bottles were suspended in a doubled black plastic bag at a depth of 1 m. This was done because it was felt that the reflection from the foil might influence the light-bottle productivity, and because of the possibility of small holes in the foil resulting in increased respiration rates. Each light bottle had a bottle lying next to it on the rack. The terminal bottles were blanks or ballast bottles such that all of the experimental bottles were exposed to the same light conditions.

After 4 hours incubation in the rack, the bottles were taken to the laboratory where the samples were vacuum-filtered through 0.45μ

pore size membrane filters. These filters were air dried and glued to planchettes.

The filters were counted using a proportional beta counter for 10 minutes each to determine the amount of carbon-14 fixed by the plankton.

These counts were then used to determine the productivity of the samples according to the formula:

$$\text{Primary productivity} = r/R\ (C)\ (f)$$

where: r = counts per minute from the jug. This is the jug mean:

$$\frac{\text{Light bottle + light bottle}}{2} - \text{dark bottle}$$

R = (scintillation count) (0.25) (0.838) $(2.22 \times 10^6)/40,290$

0.25 = counter efficiency

0.838 = membrane adsorption

2.22×10^6 = disintegrations/minute/μc

40,290 = scintillation count/minute/μc

C = 20.4×10^3 mg of available carbon per M^3 of the lakewater

f = 1.06 (isotope correction factor)

A scintillation standard was prepared with each run. The average of the scintillation counts per minute was used for the productivity calculations for each run. These were as follows:

Run No. 1	19,885.7 cpm
Run No. 2	18,545.3 cpm

Quantitative determinations of methoxychlor in these, and all subsequent, studies were made on a gas chromatograph equipped with an electron-capture detector and a 1/8-inch by 6-foot stainless steel column packed with 5% QF1 on Varaport 30. Methoxychlor was extracted from water samples with hexane, using an extraction impeller driven by a magnetic stirrer. The hexane extracts were dried with sodium sulfate before injecting into the chromatograph. Area of peak was determined by multiplying the peak height times the width at half height.

Methoxychlor determinations for the productivity studies were made as follows. Two 300-ml samples were withdrawn from the 50 μg/l jugs.

One sample was filtered through a 0.45μ membrane filter before extraction, and the other was analyzed in its original form. The difference between the two values should represent the fraction of methoxychlor adsorbed to, or incorporated in, biotic forms.

Temperature profiles were run daily during Run No. 1 and on the first day of Run No. 2 with an electric thermometer. The temperature of the water was measured at half-meter increments from 0 to 5 m and in 1-m increments from 5 to 14 m.

Secchi disc readings were only taken during Run No. 1 to obtain an estimate of the water transparency. The apparatus was lowered into the water until it just disappeared from sight. The depth was recorded in meters.

Breakdown studies. --Several authors (including Henderson et al., 1959) have indicated a lack of influence of water quality parameters, e.g., pH, alkalinity, and hardness, on the toxicity of chlorinated hydrocarbons to fishes. However, the breakdown rate of many insecticides has been found to be dependent on factors such a pH (Muhlmann and Schrader, 1957) and the presence of suitable microorganisms (Mendel et al., 1967). Therefore, our experiments on breakdown were conducted using a variety of test waters: (1) distilled water buffered with phosphate to pH 7 and 9; (2) water from Koch Warner Creek at Saline, Michigan, which serves as the water source for our experiments on chronic toxicity; (3) aged Ann Arbor tap water which had previously held fish; and (4) water from Third Sister Lake which contained plankton.

In all experiments the methoxychlor was added to the test water from stock solutions containing ethanol and sufficient Triton X-100 to assure solution of the insecticide in the water. Highest concentrations were 500 mg/l ethanol and 0.10 mg/l Triton.

Acute fish toxicity. --Four replicated 96-hour static bioassay tests were conducted; two with fathead minnows (Pimephales promelas) and two with perch (Perca flavescens). All tests were run in 10-gallon aquaria containing 30 liters of water. Ten fatheads or five perch were used in each aquarium. The perch weighed approximately 5 g each and the fatheads about 1 g. All static tests were conducted with aged Ann Arbor tap water. Continuous-flow 96-hour studies were run on fatheads in Ann Arbor tap water, and on perch in water from Koch Warner Creek. The dosing apparatus utilized in these studies was a modification of the unit described by Mount and Brungs (1967).

The results were analyzed statistically by probit analysis and logistic analysis on a computer.

Chronic fish toxicity. -- Chronic studies involved the use of four continuous-flow bioassay units, with fathead minnows and yellow perch as test fish. Fatheads were used in three units dosed at 2.0, 1.0, 0.5, 0.25, and 0.125 µg/l. The unit containing perch was dosed at 10.0, 5.0, 2.5, 1.25, and 0.625 µg/l. Each unit also had one control tank. The methoxychlor was dissolved in methanol, with Triton X-100 as a wetting agent, and injected into the units by means of a syringe injector. The injector was designed and built by a number of the staff of the Institute for Fisheries Research and proved to be very satisfactory. The final test solutions contained a range of approximately 0.4 mg/l Triton X-100 and 40 mg/l methanol in the high methoxychlor levels, to 0.026 mg/l Triton X-100 and 2.6 mg/l methanol in the low levels. The control tanks received no solvent solution. All aquaria were aerated throughout the study. Fatheads, in the chronic studies, were evaluated for dose response to growth and egg hatching success. Perch were evaluated for growth and respiratory effects.

Two bioassay units containing fatheads were dosed for 6 months, during which time we maintained water temperatures below 70 F and a photoperiod of 12 hours in order to retard spawning. At the end of 6 months we increased the temperature to 74 F and the light period to 16 hours, after which spawning commenced in about 6 weeks. Pieces of 2.5-inch plastic pipe were put in the aquaria for spawning receptacles. Ten pieces of pipe were cut in half lengthwise and stacked in the rear of each aquarium. The pieces of pipe were inspected daily for eggs. All eggs were removed from the spawning receptacles and put in hatching containers made from short pieces of 1-inch plastic pipe. A cover was made from one-half of a 1-inch plastic pipe nipple. Both the nipple and bottom of the pipe were covered with fine mesh Nitex. These hatching containers were returned to the aquarium and kept in motion by suspending them from an off-set revolving rod (approximately four revolutions per minute). A separate egg container was used for each lot of eggs, and a record was kept of the hatching success of each lot.

Two feeding plans were used to determine the amount of food given to the fish as a daily ration. The fathead minnows in units A and B were fed all of the frozen brine shrimp they would eat three times per day. When we started dosing these two units we observed that the minnows in the two highest levels of methoxychlor had exceptionally good appetites and probably ate more brine shrimp than did the fish in controls or lower levels. If the high-level fish were eating more, they could possibly compensate for any adverse effects of the methoxychlor and maintain a normal rate of growth. Consequently, to avoid this

possibility in unit D we changed the plan of feeding to 20% of the total body weight of the fish each day. A feeding of 20% of body weight is an exceptionally heavy feeding. However, the frozen brine shrimp had a high water content, and it became obvious that 20% of body weight was not supplying an adequate diet. We thus later fed the fish in unit D all they would eat, the same as in units A and B.

We started feeding brine shrimp to the yellow perch in unit C at a rate of 10% of their body weight. We found that brine shrimp is not a good food item for perch, and changed to feeding Mysis relicta at 10% of body weight. This resulted in a tremendous improvement in the growth rate of the perch.

All fish were weighed periodically, with no definite schedule. We avoided weighing the fatheads in units A and B while they were spawning to prevent possible handling mortality. The fish were weighed in a beaker of water after they had been carefully blotted dry with paper towel. All fish from an aquarium were weighed together, and the average weight per individual determined from the total weight of fish.

Respiration studies.--Perch from the long-range bioassays were used to determine the effect of continuous exposure of methoxychlor on rate of respiration. Five perch surviving from the highest level (5.0 μg/l) of treatment, five from a low level (1.25 μg/l), and five from the controls, were monitored each as a group in a tunnel respirometer similar to the one described by Brett (1962). The perch, which were dosed at 10 μg/l, died during the first month of exposure, and those dosed at 0.625 and 2.5 μg/l, died as a result of an aerator failure in the bioassay unit before they could be included in the respiration study. The tunnel respirometer allows for the measurement of oxygen consumption at any desired temperature and current velocity. Care was taken to assure that each lot of fish was handled in precisely the same manner throughout the respiration study.

All respiration measurements were made at 65 F. Each group of fish was assayed at a resting velocity (0.25 foot per second) and a working velocity (0.6 foot per second). The fish were in the respirometer for 1 week at each velocity. Velocity measurements were made with a Gurley current meter, and oxygen concentrations were monitored with a YSI meter. Fish were always put in the test chamber on Monday morning and remained there for the entire week. A determination of oxygen consumption was made once each day during the week. Each day the oxygen content was adjusted to a range of 8.0 to 9.0 mg/l. The concentration could be readily increased by bubbling oxygen into the open chamber or decreased by bubbling nitrogen. The chamber was closed and sealed each day at approximately 4:00 p.m. Oxygen

consumption was measured and the chamber was reopened the following morning after approximately 19 hours (11:00 a.m.). On Friday afternoon the fish were removed, oxygen was adjusted, and the respirometer was sealed to determine background respiration by bacteria and other microorganisms over the weekend. The background respiration values for two weekends were averaged and thus used as the mean value for the intervening week. This background value was subsequently subtracted from each daily respiration determination, in order to arrive at the rate of oxygen consumption by fish expressed as milligrams of oxygen per gram of fish per hour (mgO_2/g fish/hr).

Several respiration values were discarded when it was discovered that air bubbles had inadvertently been trapped in the respirometer. The fish were not fed while in the respirometer nor while being held over the weekend. Since each lot was used for two consecutive weeks (one week at each velocity), they were thus without food for that period of time. Each group of perch was assayed at the resting velocity during the first week, and at the faster velocity the second week.

Invertebrate toxicity. -- All bioassays were by continuous flow, using the serial dilution system and injector described earlier. Specially designed aquaria (Fig. 1) were used for the stream-dwelling insects Stenonema and Cheumatopsyche. Although a paddle-wheel system was available to produce a flowing water current in these aquaria, it was not used, as the current was maintained by the dosing rate. Pond- or pool-dwelling invertebrates were maintained in appropriate sized aquaria, e.g., midges in 1-gallon aquaria; crayfish in 5-gallon aquaria; and scuds in screened containers used for the fish eggs in the fish section of the study.

Test organisms were collected by Hester-Dendy multiple-plate samplers or dip nets, and care was taken to assure a minimum of handling.

During all bioassays the methoxychlor was added as a slurry with methanol and sufficient Triton X-100 to assure solution of the insecticide in test water. In all cases the dilution systems were operated at a flow rate of 5 liters per hour. The concentrations of methoxychlor in the aquaria were monitored by electron capture gas chromatography. Dissolved oxygen levels and water temperature were also monitored.

Most acute bioassays were run in replicate with the exception of those involving the crayfish. Mortality was measured daily for a period of 4 days. All TL_{50} values and 95% fiducial limits were calculated by both logistic and log-probit analyses. Log-probit equations were also

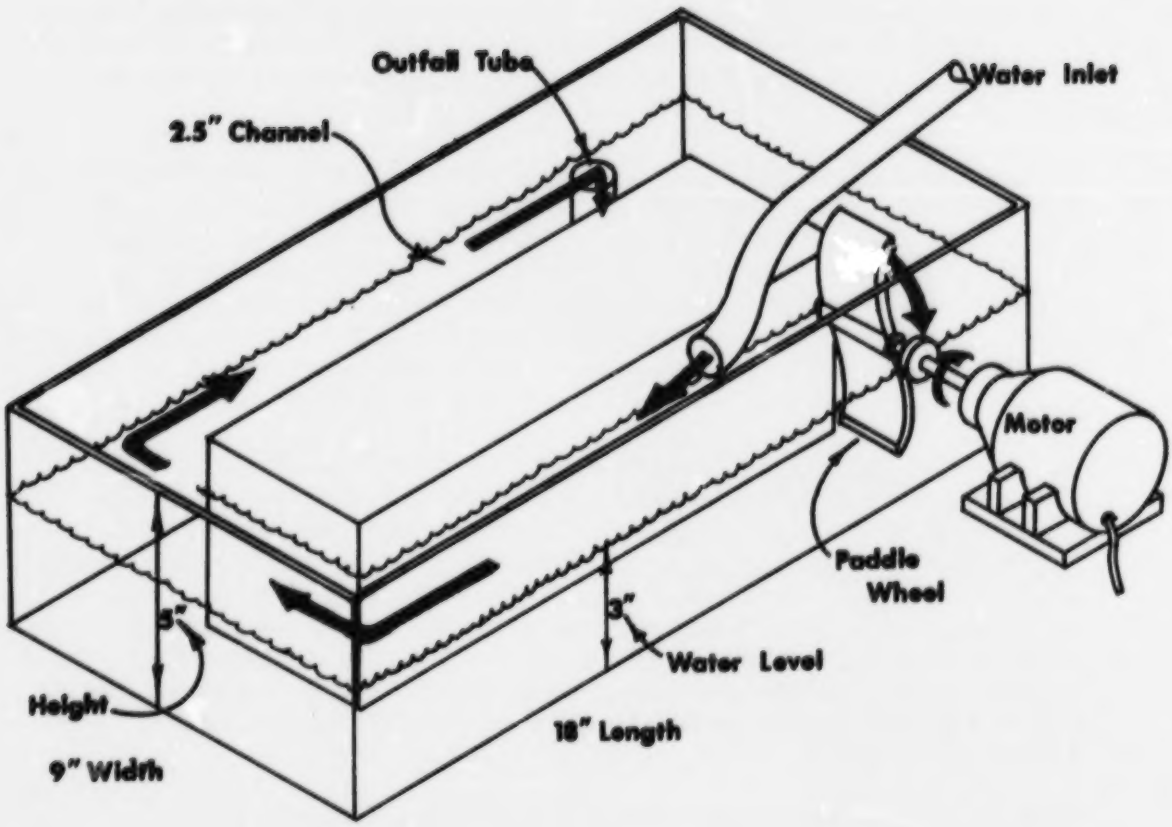

Figure 1.--Constant current aquarium used for insect toxicity studies.

generated to indicate the slope of the dose-response curve. Both types of analyses were run using computer programs by R. Hartung (personal communication) and R. Daum (1969). The data from the replicate bioassays were combined to calculate the TL_{50}'s. Mean measured pesticide concentrations were used in these dose-response analyses.

All chronic bioassays were run in replicate. All organisms were fed either by natural periphyton build up in the aquaria or leaf fragments in the case of the scuds.

Measurements of growth, mortality, and emergence were recorded during the 1-month period of the bioassays. Growth was measured both by counting the number of exuvia daily in the case of the mayflies, and by changes in the mean length of all species, exclusive of the scuds, over the 28-day period. Length, and diameter at midpoint, were measured by a modified approach to that used by Martin (1967) to measure juvenile salmon. Ectachrome slides were taken of each test group of insects initially and at 2-week intervals. The insects were photographed in a petri dish placed on a millimeter grid paper above a light source. These slides presented a permanent record of insects of each test chamber, and when projected on a screen they provided relative measurements with greater precision. Not all the necessary corrections as to the thickness of the petri dish and the depth of the water in the dishes were made, but these conditions plus the focal length of the camera were kept constant. Corrections were made for the differences in the projection measuring.

There were, of course, losses of all insect species from initial stocked populations due to mortality and emergence. A cutoff length value was therefore arbitrarily assigned to indicate a size beyond which the organism would pupate or emerge, to prevent consistent negative growth through loss of larger organisms. The length of organisms beyond this species specific length were not counted in the growth measurements. Losses due to mortality were not considered except in the cases of the higher dose levels (2.0 and 1.0 μg/l) where the initial population size was greatly reduced through mortality. Growth was not considered at these dosages because of the marked loss in sample size.

The differences in mean length were converted to wet weight in milligrams using a similar formula to that of Hynes and Coleman (1968). For the purpose of calculating volumes, Cheumatopsyche and C. tentans were considered cylindrically shaped, and Stenonema was considered to be shaped like a cylinder halved longitudinally. The measured radius at midpoint was also used instead of the assumed proportion of the length.

Screens were placed over all aquaria to collect emerging adults. However, some adults did escape, presumably through the hole cut for the dosed water supply.

Emergence, pupation, and mortality were recorded daily as were the number of exuvia. The accuracy of counting exuvia decreased as the test continued due to a buildup of an iron precipitate from the well water. Although the aquaria were cleaned thoroughly midway through the experiment, the heavy precipitate prevented adequate identification and collection of exuvia after the initial 2-week period. This also caused a clogging of the midge aquaria outflows which resulted in some of the aquaria overflowing, occasionally washing out adults that may have emerged.

SECTION V

RESULTS AND DISCUSSION

Primary production. --Two experiments were run using the carbon-14 technique outlined in the methods section. Run No. 1 was made on 9 and 10 July 1970, and Run No. 2 was made on 4 August, 6 August, 8 August and 10 August 1970. The data from Run No. 1 are in Table 1 and Table 2 for 9 and 10 July, respectively. It will be noted that for this run there is no duplicate of the Ethanol (ETOH) jug or the Ethanol + Triton X-100 (ETOH + Triton) jug. This was an inadvertent error made during the dosing of the jugs after they had been placed in the lake. Also, there is no production indicated for jugs 7 and 8 on 9 July. The counts for these two jugs indicate that the series of subsamples from these jugs were not injected with carbon-14 through operator error. Due to problems with counting, this run was terminated early. The information obtained was used to aid in the design of Run No. 2.

Data from Run No. 1 indicate an effect from the ETOH treatment on both days. However, there was only one jug with this treatment because of a dosing error. Although there seems to be a dose effect from the methoxychlor, no effect was present that could not be assigned to the ETOH.

Run No. 2 consisted of two experiments: a 2 X 2 design to test the effect of the ETOH and Triton, and a one-way design to test the effect of methoxychlor with the ETOH + Triton as a control.

The data from Run No. 2 are presented in Tables 3-6 for 4, 6, 8, and 10 August, respectively, and a summary of all data is given in Table 7. In the 2 X 2 design with the ETOH, Triton, and ETOH + Triton combinations, an analysis of variance was run to see if any of the combinations significantly depressed productivity because visual inspection of the data lead one to believe that the ethanol had a depressing effect on productivity. The analysis of variance supported the above hypothesis on 8 August 1970 (Table 5) and on 10 August 1970 (Table 6) as follows: on 8 August 1970, the mean of the experimental units that did not receive ETOH was 64.2500 as compared to the mean of the experimental units that did receive ETOH of 49.8000. On 10 August 1970, this comparison of means was 45.0500 to 17.8250. The analysis of variance gave an F value of 8.0678 (0.95 significance) for 8 August 1970, and an F value of 24.6866 (0.99 significance) was obtained for 10 August 1970. No explanation can be given for the lack of effect on the runs of 4 and 6 August 1970. It will be recalled that the effect was noted after 24 hours during Run No. 1. A possibility could be a different community of organisms during the two runs.

Table 1.--Primary production data for 9 July 1970 expressed as counts per minute

[Treatments were: Control, ETOH, Triton, and Methoxychlor (Meth) at 1, 10, and 50 $\mu g/l$]

Jug No.	Treatment	Light bottle	Light bottle	Dark bottle	Factor r [1]	Primary production [2]
11	Control	141.6	244.0	20.2	172.6	16.2
12	"	133.5	297.7	23.3	192.2	18.1
2	ETOH	81.3	90.7	27.5	58.5	5.5
5	ETOH + Triton	249.1	225.2	31.0	206.2	19.4
6	Triton	142.0	167.6	17.0	137.8	12.9
8	"	2.4	2.9	2.8	...	...
3	Meth-1	94.8	81.0	25.2	64.7	6.1
9	"	171.9	181.5	20.0	156.7	14.7
4	Meth-10	96.5	113.6	21.4	83.7	7.9
7	"	2.3	2.5	3.9	...	...
1	Meth-50	55.4	97.4	40.2	36.2	3.4
10	"	113.4	169.0	19.2	122.0	11.5
Lake	...	690.4	758.3	63.7	660.7	62.0

[1] r = the mean of the two light bottles, minus the value for the dark bottle.

[2] The formula for primary production is

$$\text{Prim. Prod.} = \frac{r}{R} C f$$

where Primary Production is measured as mg carbon/M^3/4 hours

$$R = \frac{(\text{Scintillation count})(0.25)(0.838)(2.22 \times 10^6)}{40,290}$$

$$C = 20.4 \times 10^3$$

$$f = 1.06$$

For tests on 9 and 10 July, Scintillation count was 19,885.7 cpm.

For tests on 4, 6, 8 and 10 Aug (Tables 3-6) Scintillation count was 18,545.3 cpm.

Table 2.--Primary production data for 10 July 1970 expressed as counts per minute

[Treatments were: Control, ETOH, Triton, and Methoxychlor (Meth) at 1, 10, and 50 μg/l]

Jug No.	Treatment	Light bottle	Light bottle	Dark bottle	Factor[1] r	Primary production[2]
11	Control	62.6	176.9	29.3	90.5	8.5
12	"	54.9	151.2	49.3	53.8	5.1
2	ETOH	71.4	48.8	23.1	37,0	3.5
5	ETOH + Triton	94.5	107.4	18.0	82.9	7.8
6	Triton	128.7	142.7	28.1	107.6	10.0
8	"	154.9	150.1	24.1	128.4	12.1
3	Meth-1	56.6	68.8	21.2	41.5	3.9
9	"	146.3	134.9	22.3	118.3	11.2
4	Meth-10	62.4	76.0	26.2	43.0	4.1
7	"	104.7	105.2	25.2	79.7	7.5
1	Meth-50	55.9	41.7	22.7	26.1	2.5
10	"	68.9	95.4	21.4	60.8	5.7
Lake	...	327.3	294.9	61.2	249.9	23.5

[1] [2] See footnotes to Table 1.

Table 3.--Primary production data for 4 August 1970 expressed as counts per minute

[Treatments were: Control, ETOH, Triton, and Methoxychlor (Meth) at 1, 10, and 50 μg/l]

Jug No.	Treatment	Light bottle	Light bottle	Dark bottle	Factor r [1]	Primary production [2]
4	Control	226.3	261.7	11.0	232.5	23.5
7	"	332.7	372.2	16.8	335.7	33.9
5	ETOH	311.6	305.0	16.9	291.4	29.4
11	"	320.4	364.9	16.3	326.4	32.9
3	Triton	249.1	194.1	15.1	206.5	20.8
13	"	394.4	427.0	22.6	388.1	39.2
1	ETOH + Triton	142.6	115.2	8.1	120.8	12.2
12	"	435.1	470.0	26.6	426.0	43.0
2	Meth-1	195.6	188.8	11.1	181.1	18.3
10	"	386.5	369.5	29.3	347.7	35.1
6	Meth-10	317.3	339.0	18.2	310.0	31.3
9	"	306.0	247.1	3.6	273.0	27.5
8	Meth-50	243.0	175.6	20.8	188.5	19.0
14	"	284.0	347.9	18.3	297.7	30.0
Lake	...	706.5	667.6	69.9	617.2	62.3

[1] [2] See footnotes to Table 1.

Table 4.--Primary production data for 6 August 1970 expressed as counts per minute

[Treatments were: Control, ETOH, Triton, and Methoxychlor (Meth) at 1, 10, and 50 μg/l]

Jug No.	Treatment	Light bottle	Light bottle	Dark bottle	Factor r [1]	Primary production [2]
4	Control	273.9	257.3	19.4	246.2	24.8
7	"	295.1	333.5	15.7	298.6	30.1
5	ETOH	338.5	354.0	14.3	332.0	33.5
11	"	460.6	419.2	34.9	405.0	40.9
3	Triton	320.4	390.2	6.3	349.0	35.2
13	"	437.1	474.3	33.0	422.7	42.7
1	ETOH + Triton	235.3	314.9	12.5	262.6	26.5
12	"	355.7	411.7	26.6	362.1	36.5
2	Meth-1	268.7	180.7	21.8	200.4	20.2
10	"	409.6	400.6	19.9	385.2	38.9
6	Meth-10	224.7	217.0	21.5	199.4	20.1
9	"	327.1	289.1	20.0	288.1	29.1
8	Meth-50	254.6	231.9	20.7	222.6	22.5
14	"	168.8	271.5	25.8	194.4	19.6
Lake	...	647.9	647.3	78.6	569.0	57.4

[1] [2] See footnotes to Table 1.

Table 5.--Primary production data for 8 August 1970 expressed as counts per minute

[Treatments were: Control, ETOH, Triton, and Methoxychlor (Meth) at 1, 10, and 50 μg/l]

Jug No.	Treatment	Light bottle	Light bottle	Dark bottle	Factor[1] r	Primary production[2]
4	Control	663.8	556.9	20.8	608.3	61.4
7	"	691.4	668.8	23.1	657.0	66.3
5	ETOH	497.2	564.2	23.8	506.9	51.1
11	"	501.4	549.8	29.2	496.6	50.1
3	Triton	599.8	602.1	18.2	599.1	60.4
13	"	718.7	702.3	27.4	683.1	68.9
1	ETOH + Triton	439.8	408.4	26.9	397.2	40.1
12	"	619.8	571.5	22.2	573.4	57.9
2	Meth-1	488.0	502.8	19.1	475.7	48.0
10	"	608.2	595.9	21.5	599.9	58.6
6	Meth-10	517.4	576.9	28.1	519.1	52.4
9	"	598.2	605.3	18.1	583.6	58.9
8	Meth-50	340.4	282.8	25.6	286.0	28.9
14	"	384.0	373.0	29.4	349.1	35.2
Lake	...	723.2	717.5	71.3	649.1	65.5

[1] [2] See footnotes to Table 1.

Table 6.--Primary production data for 10 August 1970 expressed as counts per minute

[Treatments were: Control, ETOH, Triton, and Methoxychlor (Meth) at 1, 10, and 50 μg/l]

Jug No.	Treatment	Light bottle	Light bottle	Dark bottle	Factor r [1]	Primary production [2]
4	Control	469.4	393.8	16.7	414.9	41.8
7	"	519.0	492.9	15.5	490.4	49.5
5	ETOH	199.8	180.3	19.2	170.8	17.2
11	"	203.2	210.4	27.6	179.2	18.1
3	Triton	370.3	374.5	17.3	355.1	35.8
13	"	551.4	543.9	21.7	526.0	53.1
1	ETOH + Triton	151.4	132.7	17.9	124.2	12.5
12	"	272.2	251.1	28.6	233.0	23.5
2	Meth-1	234.6	251.7	17.6	225.6	22.8
10	"	299.9	310.3	30.4	274.7	27.7
6	Meth-10	259.5	278.8	22.4	246.8	24.9
9	"	293.0	283.3	17.7	270.4	27.3
8	Meth-50	250.5	243.0	19.1	227.6	23.0
14	"	300.8	303.8	25.0	277.3	28.0
Lake	...	683.6	680.1	75.6	681.8	68.8

[1] [2] See footnotes to Table 1.

Table 7.--Summary of primary production data from two studies of the effects of ethanol, Triton, and methoxychlor
[Treatments were Control, ETOH, Triton, methoxychlor at 1, 10 and 50 $\mu g/l$, and lake]

Date, 1970	Treatments							
	Control	ETOH	Triton	ETOH + Triton	Meth -1	Meth -10	Meth -50	Lake
Run #1								
9 July	17.2	5.5	12.9	19.4	10.4	7.9	7.5	62.0
10 July	6.8	3.5	11.1	7.8	7.6	5.8	4.1	23.5
Mean	12.0	4.0	12.0	13.6	9.0	6.9	5.8	42.8
Run #2								
4 Aug	28.7	31.2	30.0	27.6	26.7	29.4	24.5	62.3
6 Aug	27.5	37.2	39.0	31.5	29.6	24.6	21.1	57.4
8 Aug	63.9	50.6	64.7	49.0	53.3	55.7	32.1	65.5
10 Aug	45.7	17.7	44.5	18.0	25.3	26.1	25.5	68.8
Mean	41.5	34.2	44.6	31.5	33.7	34.0	25.8	63.5

The production figures seem to indicate an initial depressing effect due to the methoxychlor at the 50 μg/l level on 4, 6, and 8 August, with slight stimulation of productivity on 10 August. The stimulatory effect is noted at the 1- and 10-μg/l levels in the data for 8 and 10 August.

The summary of the productivity data is presented in Table 7. Run No. 1 shows a short-term dose effect due to the methoxychlor when compared to the ETOH + Triton control. Run No. 2 shows an initial depressing effect at the 50-μg/l level lasting for 5 days, followed by a stimulatory effect in 7 days.

Methoxychlor levels, monitored throughout the productivity studies, tended to give differing results for the two runs. In Run No. 1 (Fig. 2) the unfiltered sample remained virtually unchanged for 2 days, then dropped rapidly to approximately one-half of the initial value by the 4th day. The concentration then changed little throughout the remainder of the experiment. The filtered samples decreased more consistently than did the unfiltered samples. The half life of the methoxychlor is approximately 6.5 days for the unfiltered material and approximately 4.6 days for the filtered material.

Figure 3 presents the data from Run No. 2, and illustrates the lack of an induction period prior to the start of degradation of the methoxychlor. The decay of the pesticide in the unfiltered material, while starting more rapidly than in Run No. 1, does not decay to the extent that was evident in the first run. The filtered samples are different from Run No. 1, showing an increase in concentration after an initial drop in concentration. This could indicate early metabolism, or adsorption, by the biota followed by death, decay and release of the methoxychlor.

It appears that the level of methoxychlor stabilized after a period of 7 days, in both filtered and unfiltered samples, at a level approximately 75% of the initial concentration. If the line for the unfiltered material in Figure 8 was projected, it would indicate a half life of about 18 days. The data for the filtered samples indicate an initial drop with a half life of approximately 4-5 days followed by an increase from the lower level.

Temperature profiles conducted during the productivity studies are shown in Figure 4. The lake was thermally stratified with the thermocline starting at approximately 2.5 m and extending to a depth of 8 m. The range of temperatures during the study is indicated in Figure 3 by the cross-hatched area. The temperature at the incubation depth (1 m) ranged from 23.5 C to 26.5 C during the period studied.

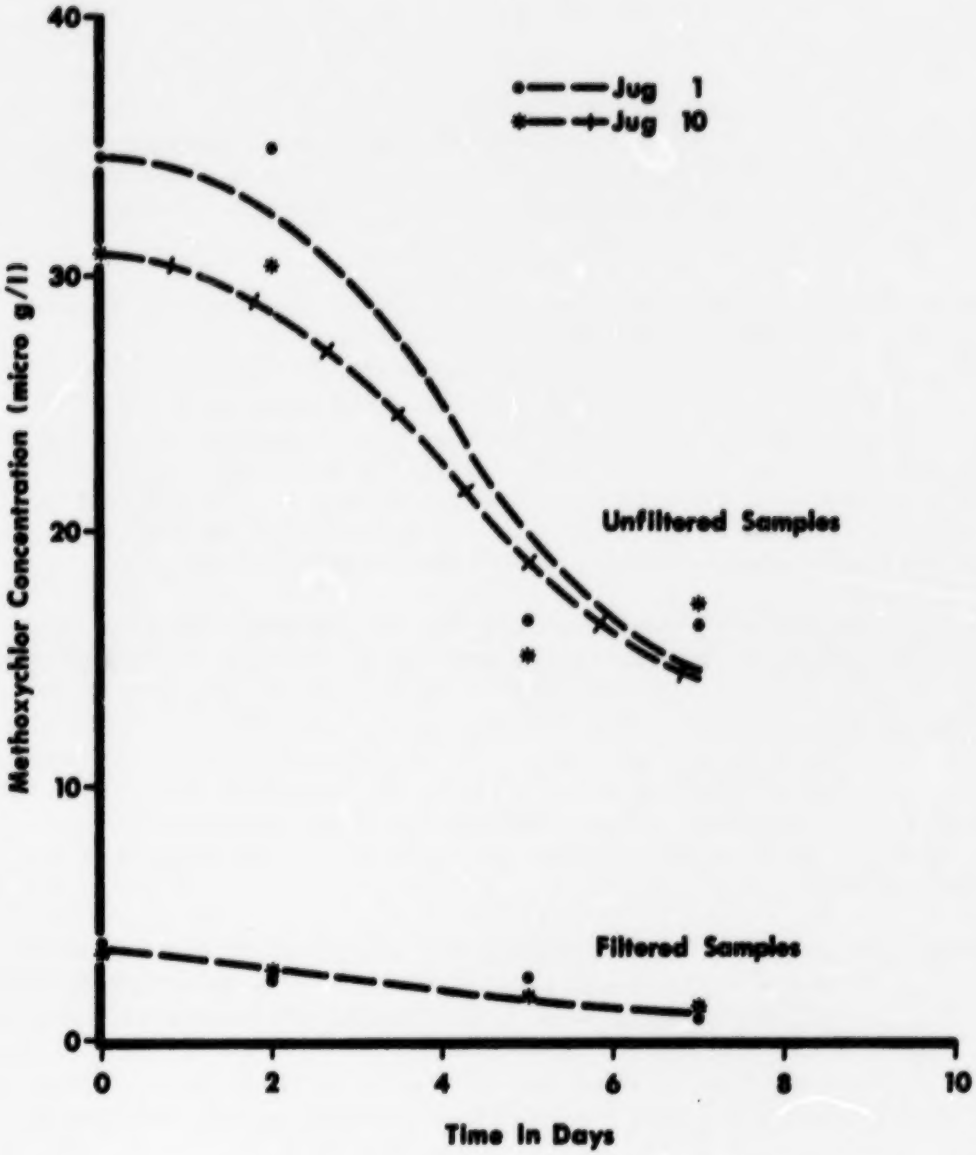

Figure 2.--Methoxychlor concentrations in test water measured during 7-day productivity study

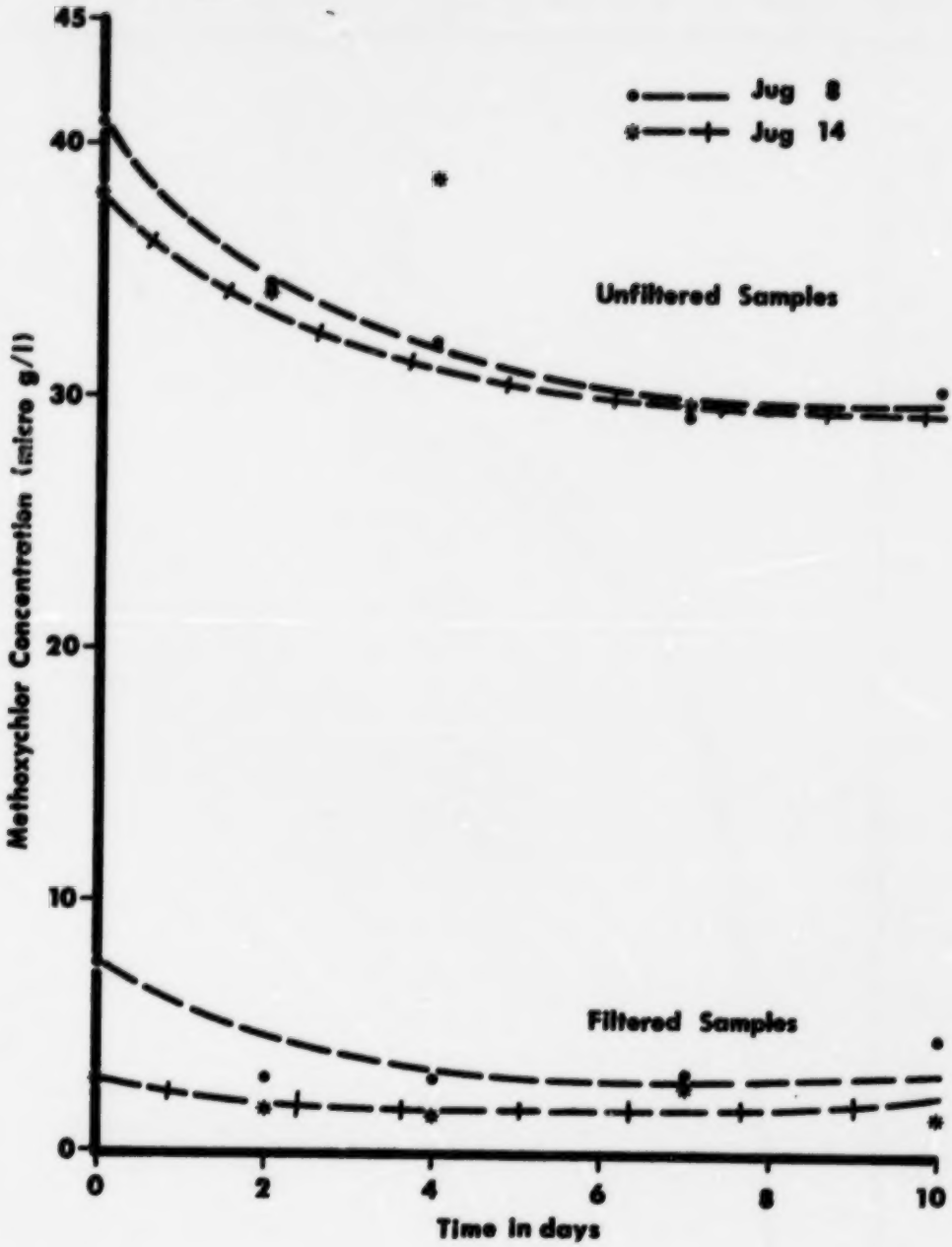

Figure 3.--Methoxychlor concentrations in filtered and unfiltered water samples measured during 10-day productivity study

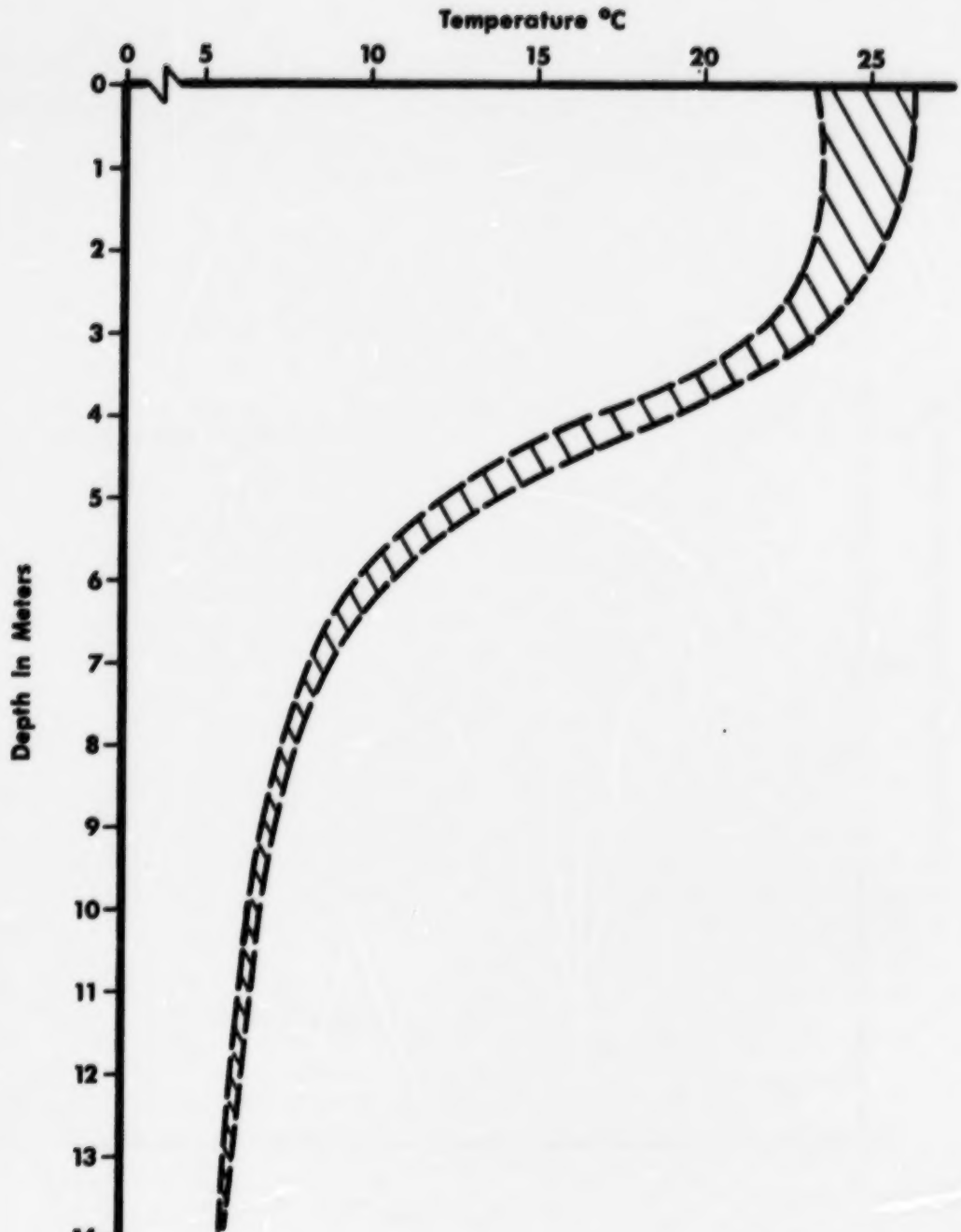

Figure 4.--Temperature profiles in Third Sister Lake during productivity studies

Breakdown studies. --Table 8 contains the chemical data from the various water supplies used for breakdown studies.

Figure 5 shows the rate of breakdown of methoxychlor in distilled water in relation to pH. As can be seen from the figure, the half life of the compound had not been attained in 220 days. The half life estimated from these data is 270 days. Hydrogen ion concentration within the range studied (pH 7-9) had no effect on the breakdown rate. Figure 6 depicts the rate of breakdown in aged Ann Arbor tap water which had previously held fish. The half life in this case was 8 days. The results from two experiments conducted with water from Koch Warner Creek are shown in Figure 7; here, in each test, two different loss rates are apparent in the data. The initial high rate was presumably due to the adsorption of methoxychlor on particles which settled and were missed in the sampling procedure. This hypothesis is supported by the study (Fig. 8) conducted with Third Sister Lake water, where in two experiments the majority of the methoxychlor was located in the particulate fraction. Measured half life was different in the two experiments; it was 7 days in the June experiment, and (by extrapolation) 18 days in the July trial.

Table 8. --Chemical characteristics of test waters

Water source	pH	Alkalinity (mg/l)	Hardness (mg/l)	Temperature (°C)
Koch Warner Creek	8.2	180	400	20 ± 2
Third Sister Lake	8.5	60	80	24 ± 1
Distilled	7 and 9	1	1	20 ± 2
Ann Arbor tap water, aged	7.0	40	60	20 ± 2

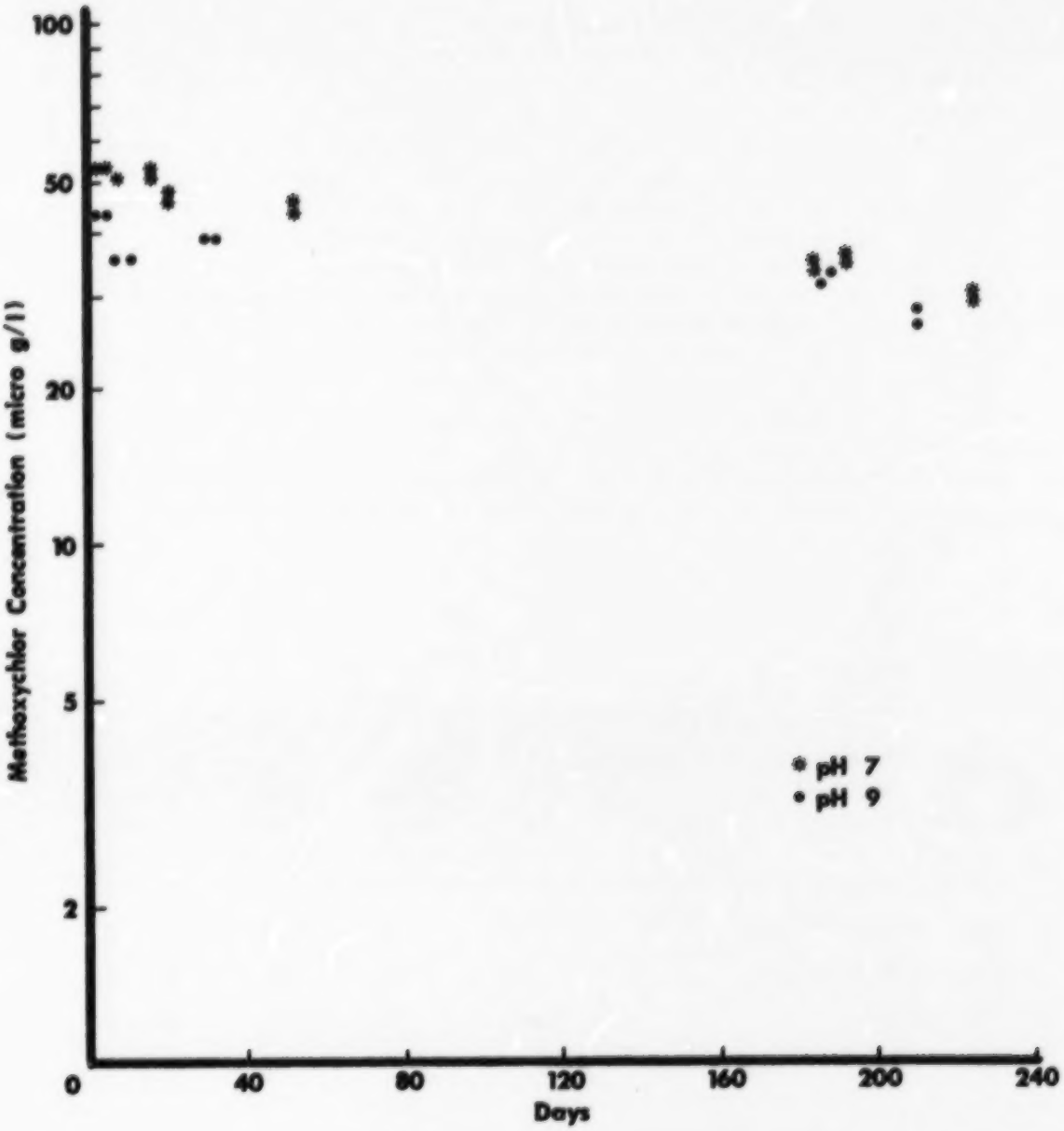

Figure 5.--Concentration of methoxychlor in replicated samples of buffered distilled water at pH 7 and pH 9 during 220 days of hydrolysis

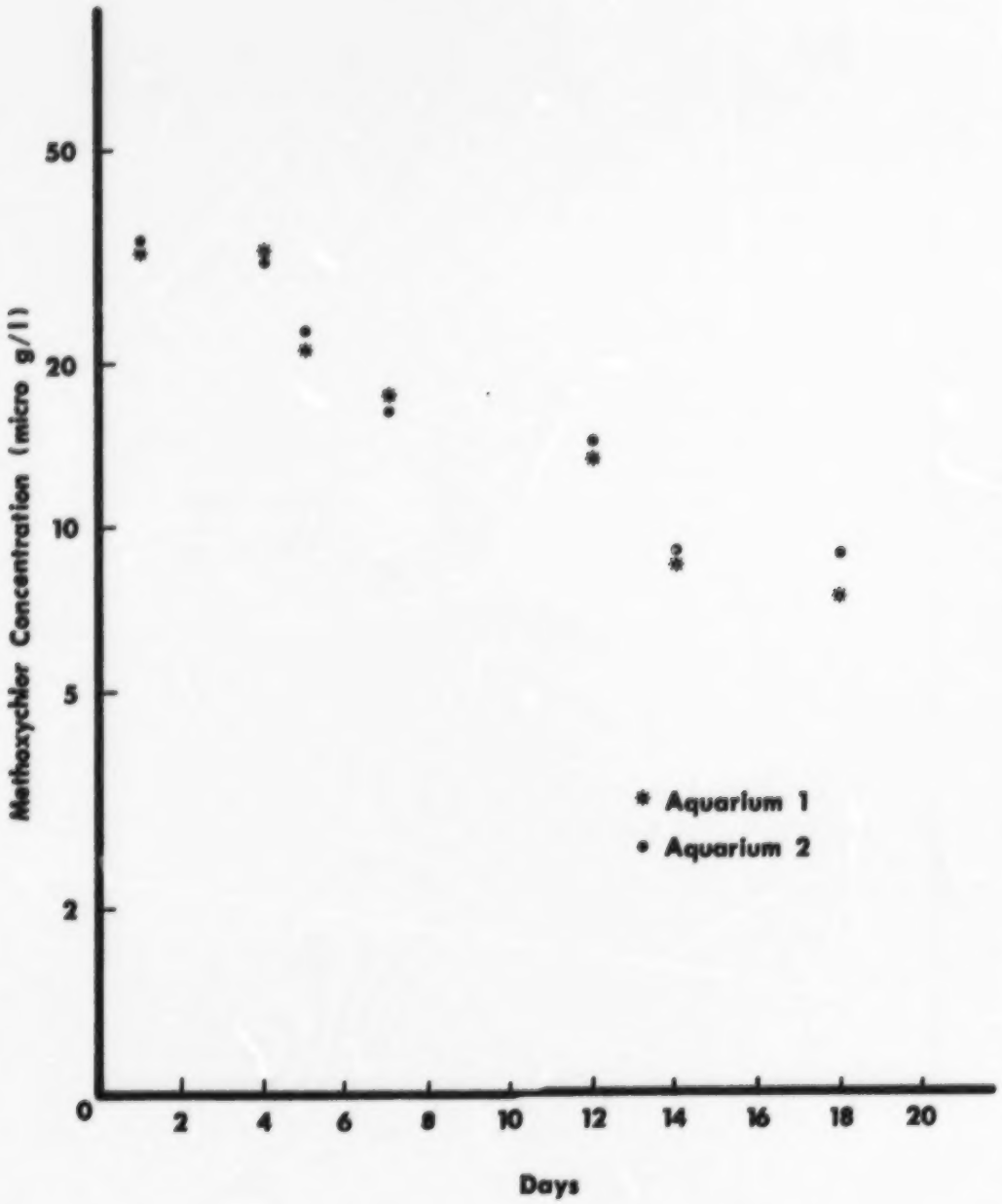

Figure 6.--Concentration of methoxychlor in replicated samples of aged Ann Arbor tap water, which had previously held fish, during 18 days of breakdown

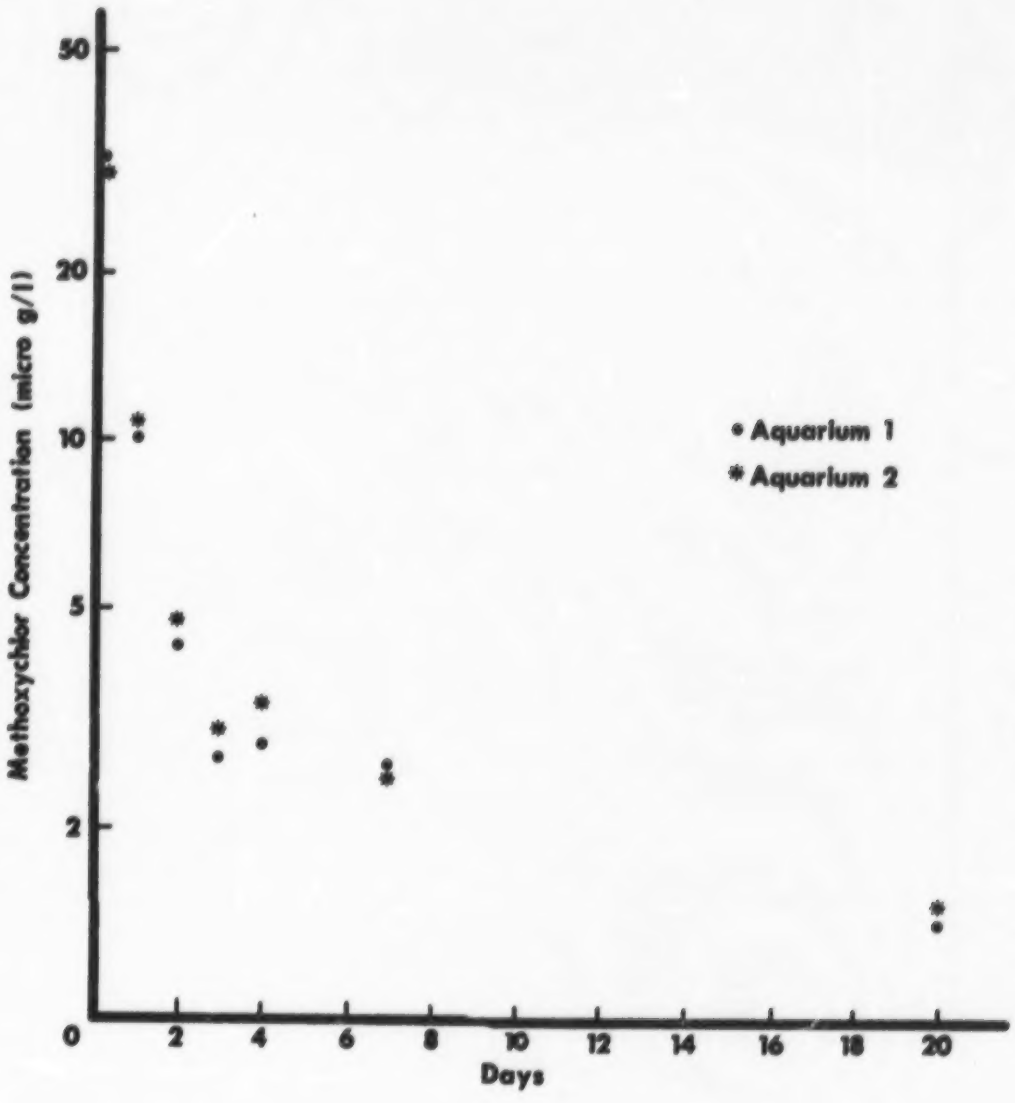

Figure 7.--Concentration of methoxychlor in replicated samples of Koch Warner Creek water during 20 days of breakdown

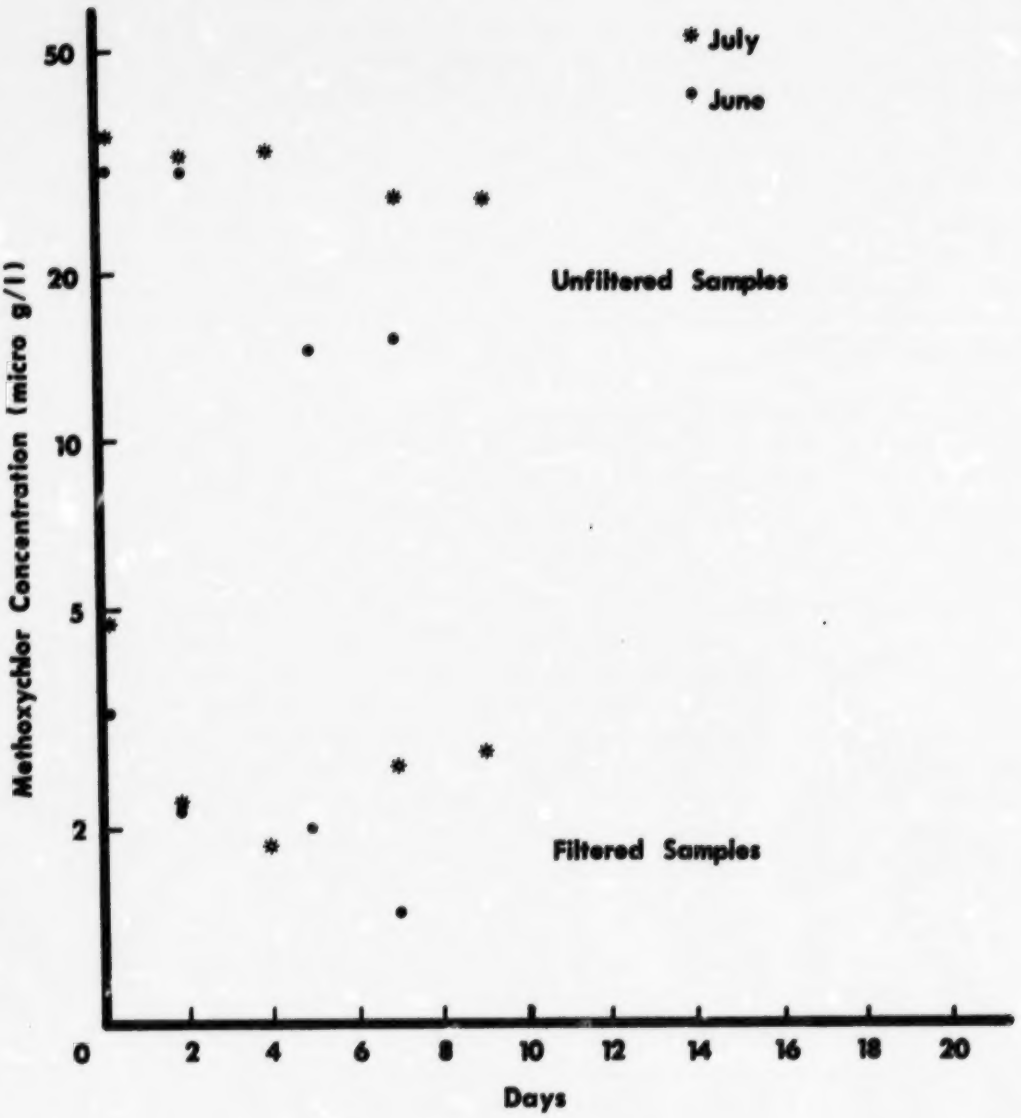

Figure 8.--Concentration of methoxychlor in filtered and unfiltered samples of Third Sister Lake water during 10 days of breakdown

Acute fish toxicity. --The static 96-hour TL_{50} values obtained during the experiments were 7.5 μg/l for fatheads, and 30.0 μg/l for perch. There are sizable differences in susceptibility of fish species to hydrocarbon insecticides. However, the results from static tests particularly with larger fish are often suspect. Our static tests on perch substantiate the weakness of these tests, as shown in Table 9, where the nominal dose and the measured concentrations of methoxychlor are given. It is obvious that the perch were exposed to the nominal concentration for a very short period of time.

Figure 9 shows the change in TL_{50} with time during the continuous-flow test for the fathead minnow. These results are averages of two replicate tests, involving two test chambers with ten fish per chamber at each concentration. Spot checks for methoxychlor, run on the high-dose and low-dose levels at the start and end of the experiment, indicated that the nominal doses were within ± 10% of the measured concentrations.

Replicated continuous-flow tests were also run using yellow perch with 8 fish per tank in Test No. I and 10 per tank in Test No. II. Table 10 gives a summary of the nominal doses, the measured concentrations, and the accumulative mortality during the 96-hour tests.

The mortality of test fish in the continuous-flow studies was interpreted by logistic and log-probit (Daum, 1969) analysis. The replicated tests were lumped to give one analysis for both the fathead minnows and yellow perch. Table 11 gives a summary of the analysis. The TL_{50} values were 8.54 μg/l for fatheads by logistic analysis and 8.63 μg/l by log probit. Corresponding values for perch were 20.12 μg/l and 22.20 μg/l. The perch had a much steeper slope (12.351) of dose response than the fathead minnows (4.696) indicating a broader range of toxicity to fatheads.

Table 9. --Measured concentrations of methoxychlor in 30 liters of water during the exposure of five yellow perch (approximately 50 g total weight) for 96 hours

Time (hours)	Nominal concentrations of methoxychlor (μg/l)		
	40	30	20
4	35.4	21.4	15.5
24	15.4	12.3	8.3
48	5.1	2.1	2.2
96	2.0	1.3	1.0

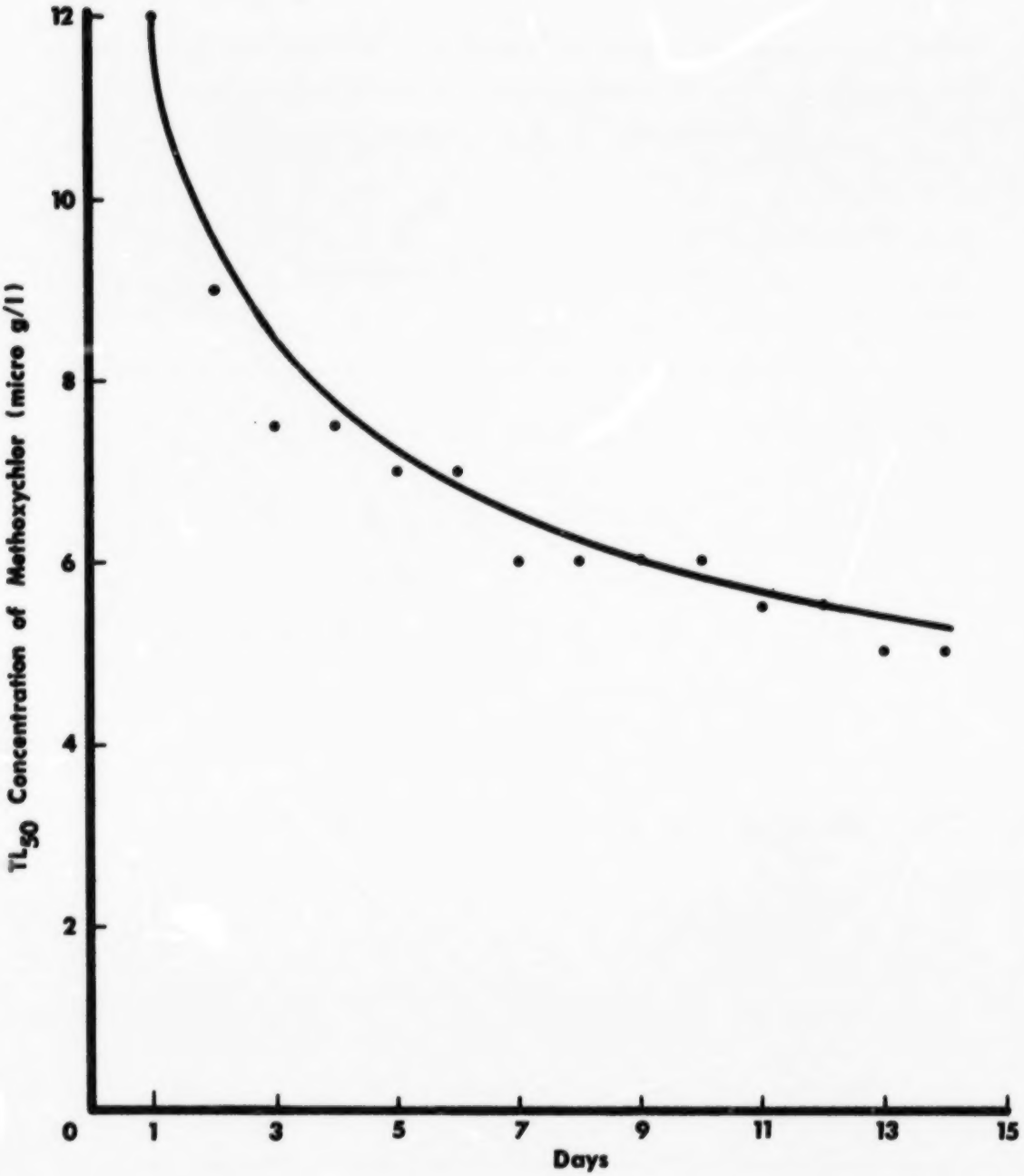

Figure 9.--Changes in median tolerance limit of methoxychlor for fathead minnows with time of exposure

Table 10.--Concentrations of methoxychlor and number of yellow perch that died during 96-hour continuous-flow toxicity study (eight perch per tank were used in Test I, and ten in Test II)

Time (hours)		Nominal concentrations of methoxychlor (μg/l)						
		Test I				Test II		
		40	20	10	5	30	15	7.5
24	Measured conc.	41.4	20.7	9.6	4.6	26.9	12.9	7.1
	Accum. mortality	7	0	0	0	5	0	0
48	Measured conc.	32.0	19.0	9.4	4.7	26.6	12.5	6.3
	Accum. mortality	8	0	0	0	9	0	0
72	Measured conc.	39.6	21.4	9.0	2.7	27.1	11.4	6.5
	Accum. mortality	8	2	0	0	9	0	0
96	Measured conc.	...	...	...	...	28.4	11.5	5.8
	Accum. mortality	8	6	0	0	9	0	0

Table 11.--Summary of dose response of fathead minnows and yellow perch to 96-hour continuous flow methoxychlor bioassay interpreted through logistic and log-probit analysis

Species	TL_{50} (µg/l) with 95% fiducial limits	Log-probit equation
Fathead minnows		
Logistic	5.30 - <u>8.54</u>* - 13.77	
Log-probit	6.42 - <u>8.63</u> - 10.62	Y = 0.604 + 4.696 X
Yellow perch		
Logistic	15.95 - <u>20.12</u> - 25.38	
Log-probit	19.69 - <u>22.20</u> - 25.59	Y = -11.630 + 12.351 X

* TL_{50} values underlined.

The difference in susceptibility of the two species is also reflected in the various TL values obtained from the probit analysis. Values for fatheads ranged from TL_{90} of 4.60 to TL_{10} of 19.33 µg/l. Values for the perch were TL_{90} of 17.49 to TL_{10} of 34.26 µg/l.

Chronic fish toxicity.--Table 12 contains data on survival and growth of fathead minnows in units A and B during 4 months of exposure to methoxychlor. The fish were reweighed on 1 April 1971, following spawning. However, all surviving fish had reached maximum size and their weight was not considered indicative of dose response. All tanks contained 14 fish at the start of the assay. There was some fish mortality in all of the aquaria; however, we are not sure that it was related to the levels of concentration of methoxychlor. In unit A there was considerable mortality at the lower levels, but almost none at the highest concentration (2 µg/l). This indiscriminate mortality in unit A made it difficult to interpret changes in the mean weight of the fish. We can show no relationship between growth of the fatheads and concentration of methoxychlor in unit A.

There was less mortality in unit B; however both growth and mortality of the fish appeared to be more closely related to methoxychlor levels. The average gain in weight of the fatheads in the two highest levels of

Table 12.--Growth and survival of fathead minnows exposed to methoxychlor at various levels of concentration

Unit	Methoxychlor concentration (μg/l)	Number of fish alive 8/5/70	Average weight (g) 4/13/70	Average weight (g) 8/5/70	Average weight gain (g)
A-6	Control	11	0.83	1.44	0.61
A-5	2.0	13	0.84	1.82	0.98
A-1	1.0	5	0.91	2.14	1.23
A-2	0.5	4	0.67	1.20	0.53
A-3	0.25	8	0.76	2.00	1.24
A-4	0.125	6	0.89	1.83	0.94
B-6	Control	13	0.66	1.46	0.84
B-5	2.0	7	0.85	1.31	0.46
B-1	1.0	8	0.84	1.31	0.47
B-2	0.5	9	0.82	1.66	0.84
B-3	0.25	10	0.72	1.42	0.72
B-4	0.125	12	0.79	1.59	0.80

methoxychlor (1 μg/l and 2 μg/l) was only one-half of the gain at the three lower levels and among the control fish.

Table 13 contains a summary of the growth and mortality of fathead minnows in unit D. These data show no relationship between growth and methoxychlor concentration.

Fish growth was converted to specific growth rates (Brown, 1957) which express growth as percent growth per unit of time. This analysis is recommended when initial sizes are not identical since growth tends to be logarithmically related to size. Table 14 contains specific growth rates of fathead minnows subjected to various concentrations of methoxychlor in units A and B. There is no indication that methoxychlor has retarded growth of any of the test fish.

Table 15 contains a summary of the growth and survival of yellow perch (unit C) subjected to various levels of methoxychlor from 11 August 1970 to 30 March 1971. All perch died at 10 μg/l, and two died at 5 μg/l. There is a striking difference in the average weight gain between the control fish and all of the test fish. There is also a striking difference in the growth of the perch after 29 December when we started feeding

Table 13.--Growth and survival of fathead minnows exposed to methoxychlor at various levels of concentration in test unit D

Concentration methoxychlor (µg/l)	Duration of exposure: 18 Aug 1970 Number of fish at start	18 Aug 1970 Average weight (g)	5 Jan 1971 Number of fish surviving	5 Jan 1971 Average weight (g)	Average weight gain (g) 18 Aug 1970-5 Jan 1971
0.125	20	0.99	20	1.51	0.52
0.25	20	0.87	18	1.17	0.30
0.50	20	0.96	20	1.34	0.38
1.0	20	0.95	19	1.33	0.38
2.0	20	0.92	15	1.23	0.31
Control	20	1.00	20	1.40	0.40

Table 14.--Specific growth rates of fathead minnows subjected to various concentrations of methoxychlor

Concentration (µg/l)	Growth interval: 13 April to 5 Aug 1970	5 Aug 1970 to 1 April 1971	Total 13 April 1970 to 1 April 1971
Unit A			
Control	0.481	0.190	0.277
0.125	0.634	0.317	0.419
0.25	0.848	0.211	0.416
0.50	0.511	0.446	0.467
1.00	0.749	0.319	0.457
2.00	0.679	0.261	0.395
Unit B			
Control	0.695	0.180	0.347
0.125	0.612	0.203	0.336
0.25	0.603	0.322	0.413
0.50	0.616	0.203	0.337
1.00	0.390	0.291	0.322
2.00	0.378	0.354	0.363

Table 15.--Average weight in grams of yellow perch[1] during exposure to methoxychlor, and average gain in weight during test periods in unit C

Concentration of methoxychlor (µg/l)	At these dates							
	11 Aug 1970		29 Dec 1970		17 Feb 1971		30 March 1971	
	Number fish	Weight	Number fish	Weight	Number fish	Weight	Number fish	Weight
Control	6	8.33	6	11.50	5	19.20	5	25.20
0.625	9	7.56	9	9.11	9	14.44	9	19.44
1.25	9	7.00	8	8.75	8	13.75	8	18.00
2.50	9	7.00	9	8.00	9	12.67	9	16.56
5.00	9	5.89	7	7.28	7	11.15	7	14.57
10.00	9	7.56	0	...	0	...	0	...

	Gain in weight during periods			
	11 Aug to 29 Dec	29 Dec to 17 Feb	17 Feb to 30 March	11 Aug 1970 to March 30 1971
Control	3.47	7.40	6.00	16.87
0.625	1.55	5.33	5.00	11.88
1.25	1.75	5.00	4.25	11.00
2.50	1.00	4.67	3.89	9.56
5.00	1.39	3.87	3.42	8.68

[1] Perch were fed frozen Artemia prior to 29 December, and frozen Mysis relicta for the remainder of the study.

Mysis. Prior to 29 December, growth seemed to be retarded equally at all levels of methoxychlor. After we improved the diet of the perch, however, there was a considerable range of effect at various levels of exposure.

The weight gains of the perch were also converted to specific growth rates which are given in Table 16. The rates for the control fish are significantly higher than any of the test fish, however the effect is not linear with dose within the levels tested.

Table 17 contains data on hatching success of fathead minnow eggs as influenced by various levels of methoxychlor. There was no spawning at 2 μg/l, and only two lots of eggs at 1 μg/l. The only enigma here is the 92% hatching success of one of these lots of eggs. Totals from tests A and B give a 66% hatching success for eggs from control fish, and a range of 29 to 37% success for eggs from treated fish. Hatching percentage was not linear with dose.

An analysis of variance indicated no significant difference in the numbers of eggs deposited per individual spawning. We had no way of knowing how many females actually spawned, or whether some spawned more than once.

We selected one lot of eggs from the control tank of unit A, put one-half of the eggs in an aquarium dosed at 2 μg/l, and left the other half in the control tank. None of the eggs hatched at 2 μg/l, whereas 81% of those left in the control tank hatched.

Table 16.--Specific growth rates of yellow perch subjected to various concentrations of methoxychlor

Concentration (μg/l)	Growth interval			Total
	11 Aug. to 29 Dec. 1970	29 Dec. 1970 to 17 Feb. 1971	17 Feb. to 30 March 1971	11 Aug. 1970 to 30 March 1971
Control	0.249	0.974	0.647	0.477
0.625	0.133	0.921	0.708	0.407
1.25	0.159	0.904	0.641	0.407
2.50	0.095	0.920	0.638	0.371
5.00	0.151	0.853	0.637	0.390
10.00*	...	...	...	...

* All fish died at 10 μg/l.

Table 17.--Spawning and hatching success of fathead minnows at methoxychlor levels of 2 μg/l and less

Data given are number of eggs (E) and percentage hatched (%H)

Unit	Concentration of methoxychlor (μg/l) ↓									
	1.0		0.5		0.25		0.125		Control	
	E	%H	E	%H	E	%H	E	%H	E	%H
A	0	0	12	100	109	11	269	31	51	0
	...	..	89	11	232	13	84	0	50	0
	...	..	58	0	37*	95	221	58	55	82
	...	..	90	0	...	..	...	..	5	100
	...	..	33	9	...	..	...	..	57	94
	...	..	144	36	...	..	...	..	17	82
	...	..	78	100	...	..	...	..	5	80
	...	..	42	83	...	..	...	..	...	..
Total	0	0	546	35	378	20	574	37	240	51
B	174	0	59	0	152	15	42	71	159	69
	89	92	11	91	141	27	88	17	364	85
	...	..	407	27	94	11	174	66	31	87
	...	..	...	..	67	90	264	13	39	85
	...	..	...	..	28	75	20	85	190	68
	...	..	...	..	262	5	22	90	46	100
	...	..	...	..	29	66	212	36	32	31
	...	..	...	..	105	64	157	43	70	94
	...	..	...	..	22	96	23	74	181	22
	...	..	...	..	37	95	...	..	...	..
Total	263	31	477	25	937	33	1,002	39	1,112	69
Total A + B	263	31	1,023	31	1,315	29	1,576	37	1,352	66

↓No eggs were spawned at a concentration of 2.0 μg/l.

Respiration studies.--Values of oxygen consumption in the respirometer by the control perch and by those exposed to two different levels of methoxychlor, are given in Table 18. A two-way analysis of variance indicated the only significant differences within these sets of data were that the fish from high level methoxychlor (5.0 μg/l) tested at the high velocity give significantly high values of oxygen consumption. The values are significantly higher (at 95% level of confidence) than those for any other combination of methoxychlor and velocity. Exposure to 5 μg/l of methoxychlor apparently caused physiological damage to the perch, resulting in a high oxygen demand when subjected to continual physical exertion.

Table 18.--Oxygen consumption in milligrams per gram of fish per hour (mg/g/hr), by yellow perch after long-term exposure to methoxychlor, measured at two rates of swimming speed

Exposure to methoxychlor	Swimming speed	
	Low velocity (0.25 ft/sec)	High velocity (0.6 ft/sec)
Control	0.1758	0.1570
	0.1717	0.1682
		0.1524
1.25 μg/l	0.1872	0.1475
	0.1406	0.1733
	0.1187	0.1842
		0.1346
5.0 μg/l	0.0943	0.3836
	0.1792	0.2179
		0.1821
		0.1556

Invertebrate toxicity.--The 96-hour TL_{50}'s, fiducial limits, log-probit equation, water temperature, and mean dissolved oxygen are given in Table 19. The TL_{50} value is flanked by its 95% fiducial limits. In some cases a non-significant regression was obtained using probit analysis. This implies that the data were not a good fit to the log-probit analysis nor the logistic analysis as both are similar. A TL_{50} value was given through the logistic analysis to approximate the true value. Because the logistic and log-probit analyses are based on similar assumptions, they yield similar TL_{50} values as is evident here. In all cases the 96-hour TL_{50} values were in the low micrograms-per-liter range. The most susceptible organism was the adult scud while the crayfish was least susceptible with 96-hour TL_{50} values of 0.75 and 7.05, respectively. The crayfish were second-year-class adults (mean carapace length, 3.3 cm) and the mayflies, midges, and caddisflies were late larval instars.

The steep slopes of the midge and crayfish log-probit line indicate that the toxicity range is narrow and thus there may be a threshold pesticide level beyond which toxicity is great. The effect of oxygen level on toxicity in crayfish is apparent as the toxicity at low dissolved oxygen levels (unaerated aquaria) is three times that of normal dissolved oxygen levels (aerated aquaria). This distinction was made as it was found that the dissolved oxygen levels were greatly depressed during the first bioassay, therefore a second bioassay was conducted, artificially aerating the aquaria. This oxygen effect was noted in other bioassays (Table 20) although there was no significant difference between dosed and control aquaria. Jensen (1965) observed increased oxygen uptake of two stonefly species under DDT stress and it has been generally accepted that this is the case.

Although it appears that age may be a factor in scud susceptibility to methoxychlor as the 96-hour TL_{50} for the young is less than that of the adult, this may be more a case of different water temperatures involved in both bioassays.

The chronic bioassay TL_{50}'s, fiducial limits, log-probit equations, temperature, and mean dissolved oxygen are given for 2-week periods in Table 21. The duration of chronic tests varied from 2 weeks to 42 days depending on the length of time the various species could be maintained in good condition. For most invertebrates the mortality decreased greatly after the second week of dosing. Insect resistance or vigor tolerance to pesticides has been reported especially for Stenonema (Grant and Brown, 1967). Although organisms had different 96-hour TL_{50}'s, they all had similar chronic TL_{50}'s for as long as the respective bioassays were conducted. Replicate bioassays were combined for the dose response analyses.

Table 19.--Results of 96-hour continuous-flow invertebrate bioassays, with recorded values of water temperature and mean dissolved oxygen, interpreted through logistic and log-probit analyses

Test organism	Water temp (° F)	Mean D.O. (ppm)	96-hr TL_{50}* µg/l (with 95% fiducial limits)			Log probit equation
Stenonema interpunctatum						
Larva						
Logistic	62±4	8.2	1.09	1.96	3.11	
Log probit			NS regression			Y = 4.013 + 3.950 X
Chironomus tentans						
Larva						
Logistic	62±4	8.2	1.19	1.62	2.30	
Log probit			1.41	1.59	1.81	Y = 3.450 + 7.716 X
Cheumatopsyche sp.						
Larva						
Logistic	54±4	6.1	2.89	3.24	3.63	
Log probit			2.42	3.26	4.34	Y = 3.554 + 2.814 X
Gammarus pseudolimnaeus						
Young						
Logistic	54±4	6.1	0.84	1.14	1.54	
Log probit			NS regression			Y = 4.873 + 1.298 X
Adult						
Logistic	37±3	7.5	0.47	0.75	1.22	
Log probit			0.41	0.61	0.98	Y = 5.579 + 2.698 X
Orconectes virilis						
Adult (unaerated)						
Logistic	60±4	1.7	0.62	2.15	3.79	
Log probit			0.41	2.15	10.61	Y = 3.131 + 5.626 X
Adult (aerated)						
Logistic	60±4	5.5	3.22	7.05	15.42	
Log probit			NS regression			None

* TL_{50} values are underlined.

Table 20.--Dissolved oxygen levels in the Stenonema sp. and Chironomus tentans continuous-flow 96-hour bioassay (Test I)

Concentration				
Replicate A				
Methoxychlor (µg/l)	9.62 ± 1.47	1.23 ± .99	0.92 ± .40	0
Oxygen (mg/l)	7.71 ± .52	8.35 ± .64	8.35 ± .21	8.41 ± .19
Replicate B				
	High conc.	Low conc.	Control	
Methoxychlor (µg/l)	8.0 ± 1.35	0.55 ± .064	0	
Oxygen (mg/l)	8.47 ± 1.02	8.49 ± .16	8.74 ± .172	

Table 21.--Results of chronic continuous-flow invertebrate bioassay interpreted at 2-week intervals through logistic and log-probit analyses

Test organism[1]	Water temp (°F)	Mean D.O. (ppm)	Interval (weeks)	TL_{50} *in µg/l (with 95% fiducial limits)			Log-probit equation
Stenonema terminatum							
Larva							
Logistic	68±4	6.0	2	0.32	**0.41**	0.51	
Log probit				0.002	**0.41**	1.44	Y = 5.515 + 1.321 X
Stenonema interpunctatum							
Larva							
Logistic	68±4	6.0	2	0.38	**0.47**	0.58	
Log probit				0.25	**0.47**	0.74	Y = 5.431 + 1.302 X
Logistic	68±4	6.0	4	0.13	**0.22**	0.38	
Log probit				NS regression			Y = 6.148 + 2.068 X
Cheumatopsyche sp.							
Larva							
Logistic	54±4	6.5	2	0.60	**0.71**	0.85	
Log probit				0.44	**0.72**	1.37	Y = 5.246 + 1.753 X
Logistic	54±4	6.5	4	0.42	**0.49**	0.57	
Log probit				NS regression			Y = 6.645 + 2.127 X
Logistic	54±4	6.5	6	0.17	**0.21**	0.26	
Log probit				0.07	**0.21**	0.33	Y = 6.926 + 2.877 X
Gammarus pseudolimnaeus							
Young							
Logistic	54±4	6.5	2	0.26	**0.30**	0.34	
Log probit				0.28	**0.33**	0.36	Y = 8.104 + 3.402 X
Logistic	54±4	6.5	4	0.23	**0.29**	0.36	
Log probit				NS regression			Y = 11.76 + 19.203 X
Logistic	54±4	6.5	6	0.20	**0.22**	0.24	
Log probit				NS regression			None

* TL_{50} values are underlined.

[1] For tests on larval Chironomus tentans, see text.

The chronic midge bioassay, while mentioned, has no data presented in Table 21 because of seemingly high mortality in the controls due to low recovery of initial test organisms (Fig. 10). This loss negates standard dose response analyses. Mulla (1969) indicates that high mortality in her bioassay controls was due to cannibalism, and the same may be true here. All recovered insects were alive in all dosed aquaria except at the two highest dose levels, 2.0 and 1.0 μg/l. It was assumed that the low recovery in these aquaria was due to pesticide induced mortality while low recovery in the control was due to cannibalism. Highest recovery of initial test organisms was in a low dosage (0.25 μg/l) aquaria.

Emergence is expressed as emergence only for Stenonema, and pupation only for Cheumatopsyche (Fig. 11), as no caddisflies emerged. Pupation and emergence are lumped for C. tentans (Fig. 10). The total emergence for a 28-day period is plotted and the two mayfly species groups are combined as are all replicate bioassays.

Interestingly the low dosage levels (0.125 or 0.25 μg/l) experienced higher, or comparable, emergence than the controls in all cases. Although the curves appear to be skewed, if dose levels were transformed to logarithmic scale as they are in standard dose response analyses, the curves would approach normal or Gaussian distributions.

Growth as expressed for Stenonema by number of exuvia per 28-day bioassay is plotted in Figure 12. As can be seen the numbers of exuvia per individual are generally dose related, as there were fewer exuvia in the highest dosage aquaria with a general linear increase as the dosing level was decreased.

Table 22 indicates the calculated growth for all species in milligrams per individual using the length measurements made at 2-week intervals. Again, as in the emergence data, the mayflies and midges seemingly have faster growth at the low pesticide levels (0.125 and 0.25 μg/l) than in the control. The growth of Cheumatopsyche is greater in the control than in all dosed samples.

In all cases there is markedly reduced growth at the 0.5 μg/l pesticide level indicating that there may be a threshold beyond which lower pesticide exposures are not inhibitory with regard to growth.

Dissolved oxygen levels were measured weekly in all mayfly aquaria after it was established that the midge aquaria maintained the same levels as their source mayfly aquaria. The mean dissolved oxygen levels corresponding to the pesticide levels ranged from 5.7 mg/l to 7.35 mg/l with a low and high individual value of 4.9 mg/l and

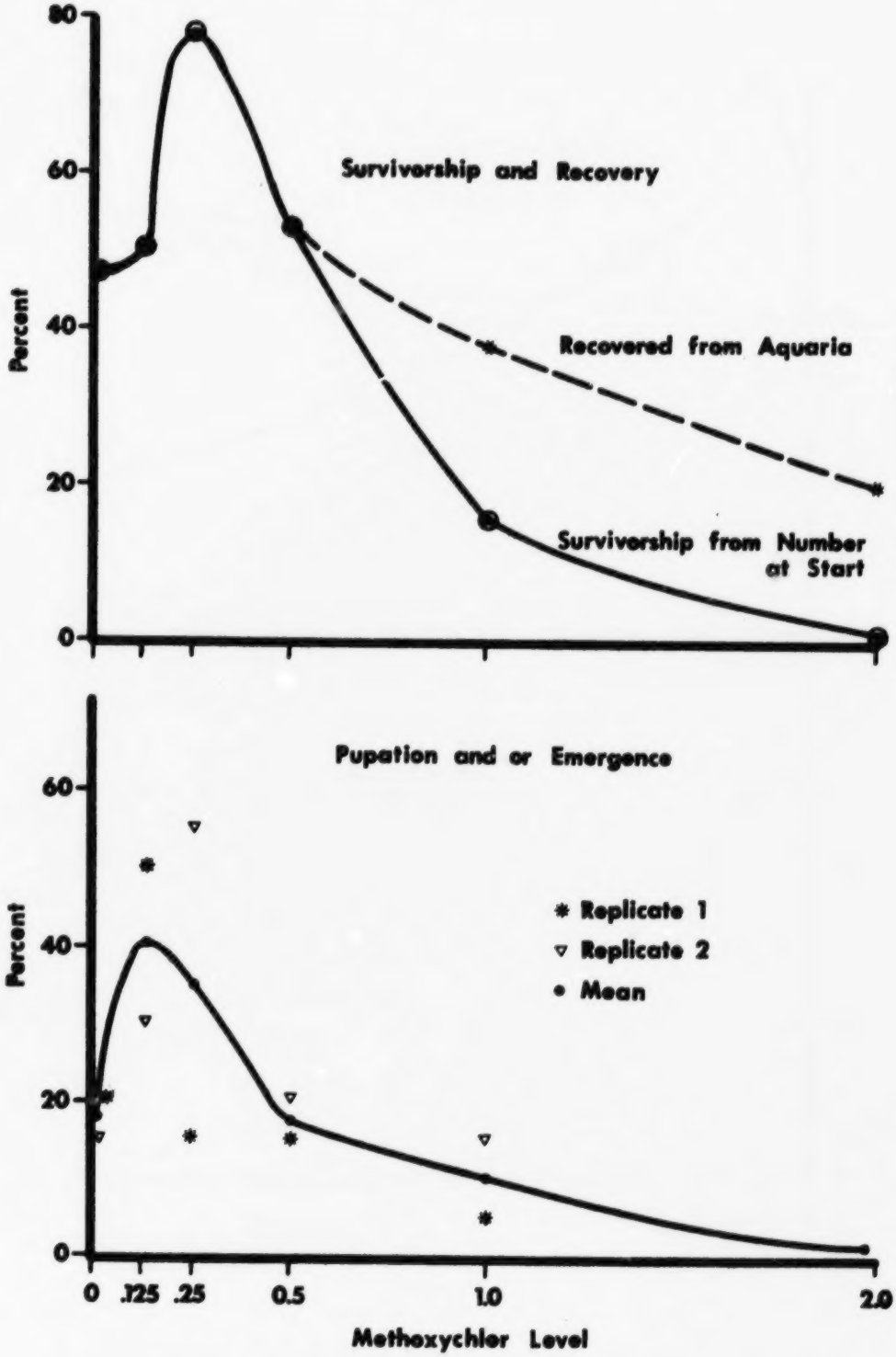

Figure 10.--Percent survivorship and recovery of Chironomidae larvae from 28-day chronic methoxychlor bioassay

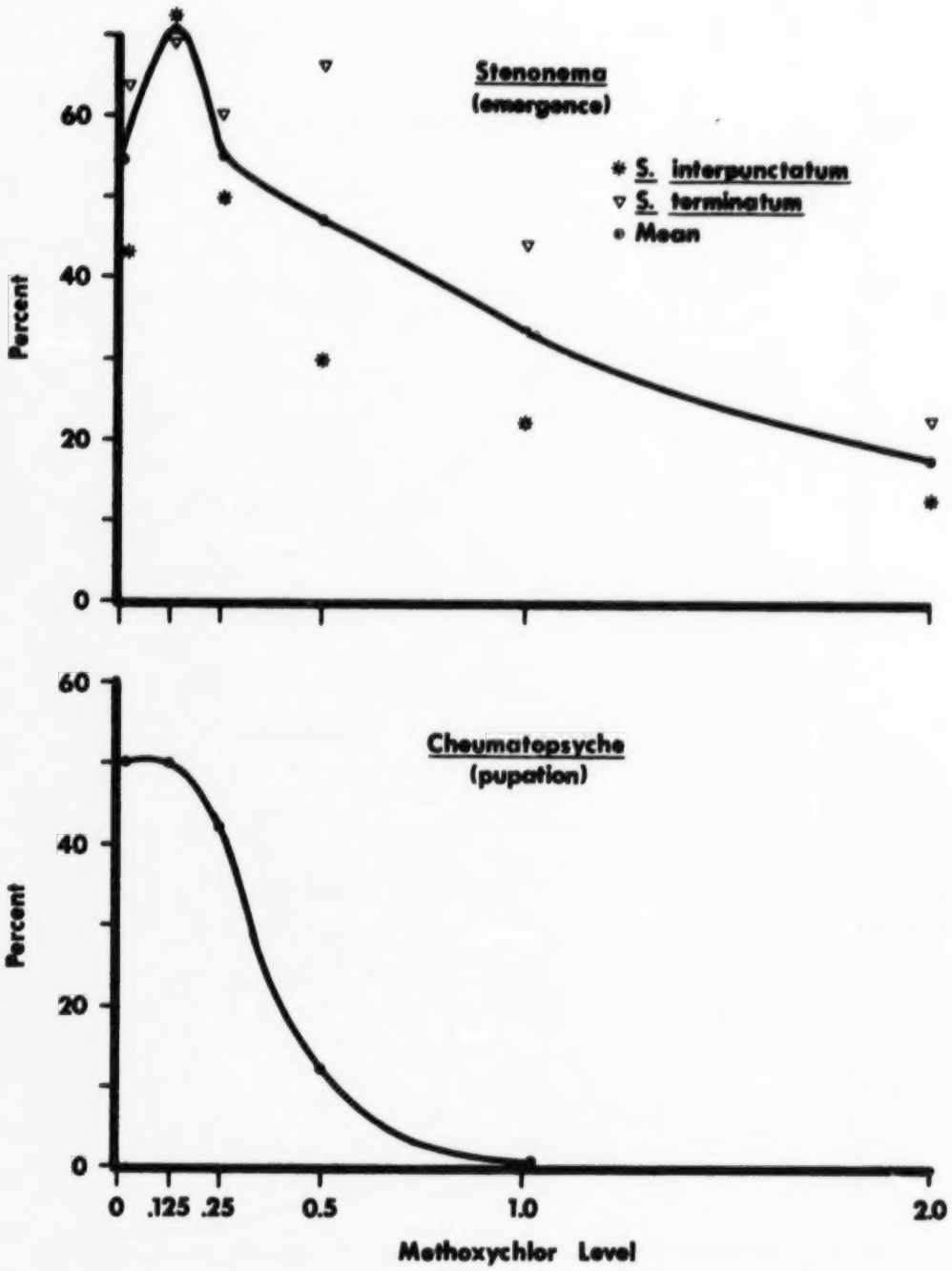

Figure 11. --Percent emergence of Stenonema and pupation of Cheumatopsyche during 28-day chronic methoxychlor bioassay

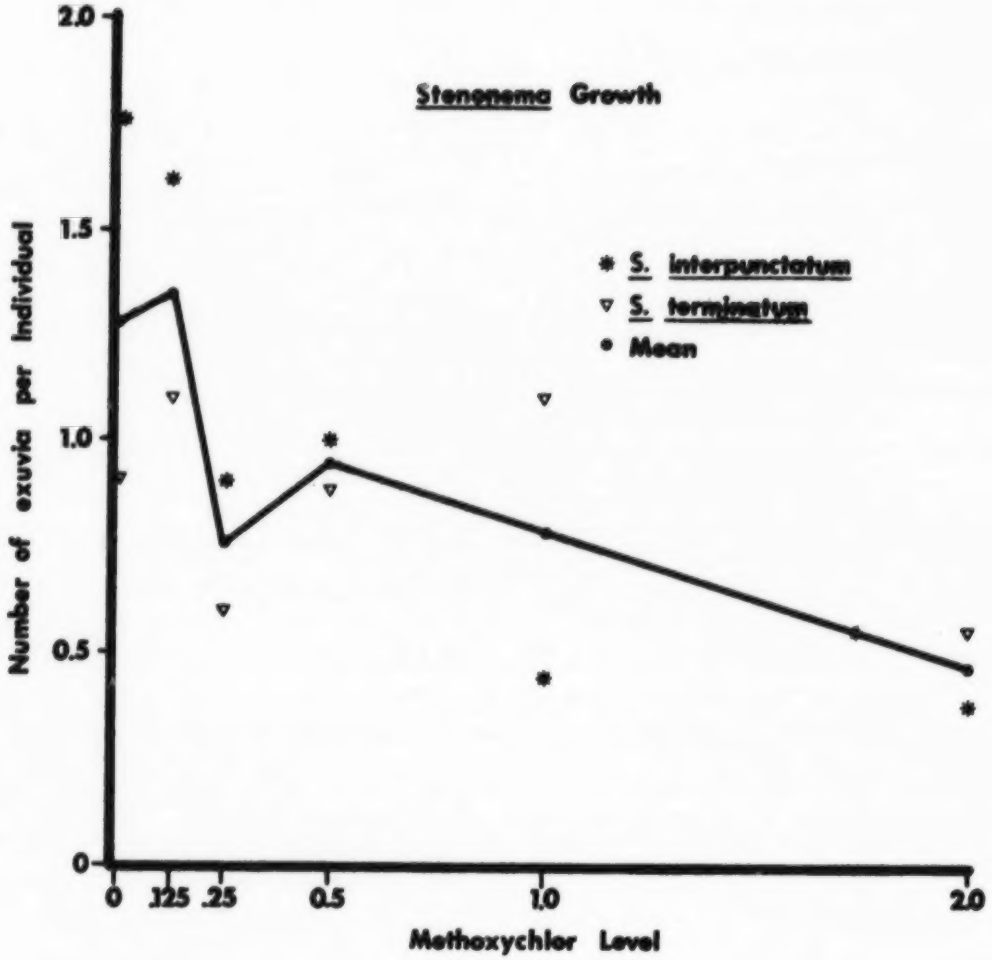

Figure 12.--Growth of Stenonema, as expressed by number of exuvia per individual, during 28-day chronic methoxychlor bioassay

Table 22.--Growth in milligrams of all insect larvae tested during chronic continuous-flow methoxychlor bioassay

Organism	Time (weeks)	Concentration of methoxychlor			
		0.5	0.25	0.125	Control
Stenonema interpunctatum group	2	-0.200	1.087	0.057	0.369
S. terminatum group	2	0 646	1.291	2.432	2.224
Mean		0.223	1.189	1.244	1.296
Chironomus tentans (Replicate 1)	2	0.487	0.932	0.773	0.600
(Replicate 2)	2	0.155	0.843	0.838	0.201
Mean		0.321	0.888	0.806	0.400
Cheumatopsyche sp.	2	0.347	0.601	0.579	1.306
	4	-0.057	0.521	0.507	1.532
Mean		0.145	0.561	0.543	1.419

8.1 mg/l, respectively. A one-way analysis of variance (ANOVA) showed the means to be significantly different at the 95% level. Paired Scheffe contrasts at the 95% level showed the mean dissolved oxygen level of the second highest pesticide level tank (5.7 mg/l) to be significantly different from that of the control tank (7.35 mg/l).

SECTION VI

STREAM CONSTRUCTION

One phase of this study consisted of the construction of six experimental streams that will constitute a permanent facility for conducting long-term sublethal pollution studies. The streams are of such size and design that fish and other aquatic fauna can be raised in a nearly natural habitat.

The streams were constructed within a millrace at the Saline Research Station of the Institute for Fisheries Research, Michigan Department of Natural Resources. About 400 feet of the millrace was divided lengthwise by a cement wall to form two streams each 18 feet wide. These streams were further divided crosswise to form six stream segments each 120 feet long. Each stream segment has a shallow riffle with a depth of about 1 foot at the head end, and then a gradual slope into a pool with a depth of 3 1/2 feet at the foot end. The crosswise dividers are cement boxes 8 feet wide and 20 feet long, and in addition to dividing the stream they serve as mixing chambers for the methoxychlor, and collection boxes for fish when draining the streams.

An electric butterfly valve controls the flow of water from a small impoundment to the streams, and excess flow can be diverted into a bypass creek.

The streams will be operated with a total of 1 cfs of water (0.5 cfs for each stream segment). The design of the streams dictates that the flow be continuous through the length of the experimental area rather than each stream having an individual water supply. This fact dictates the experimental design of the study. The two upstream sections will be control streams, the two middle sections low-level treatment, and the two downstream sections high-level treatment. Charcoal will be used to filter out the methoxychlor below the experimental sections.

An underestimate of the cost of construction of the streams resulted in extensive delays in completion. The streams were completed in the fall of 1972, and will be used for the first experiments during the summer of 1973. Figure 13 shows photographs of the streams during construction and in testing operation during the past winter.

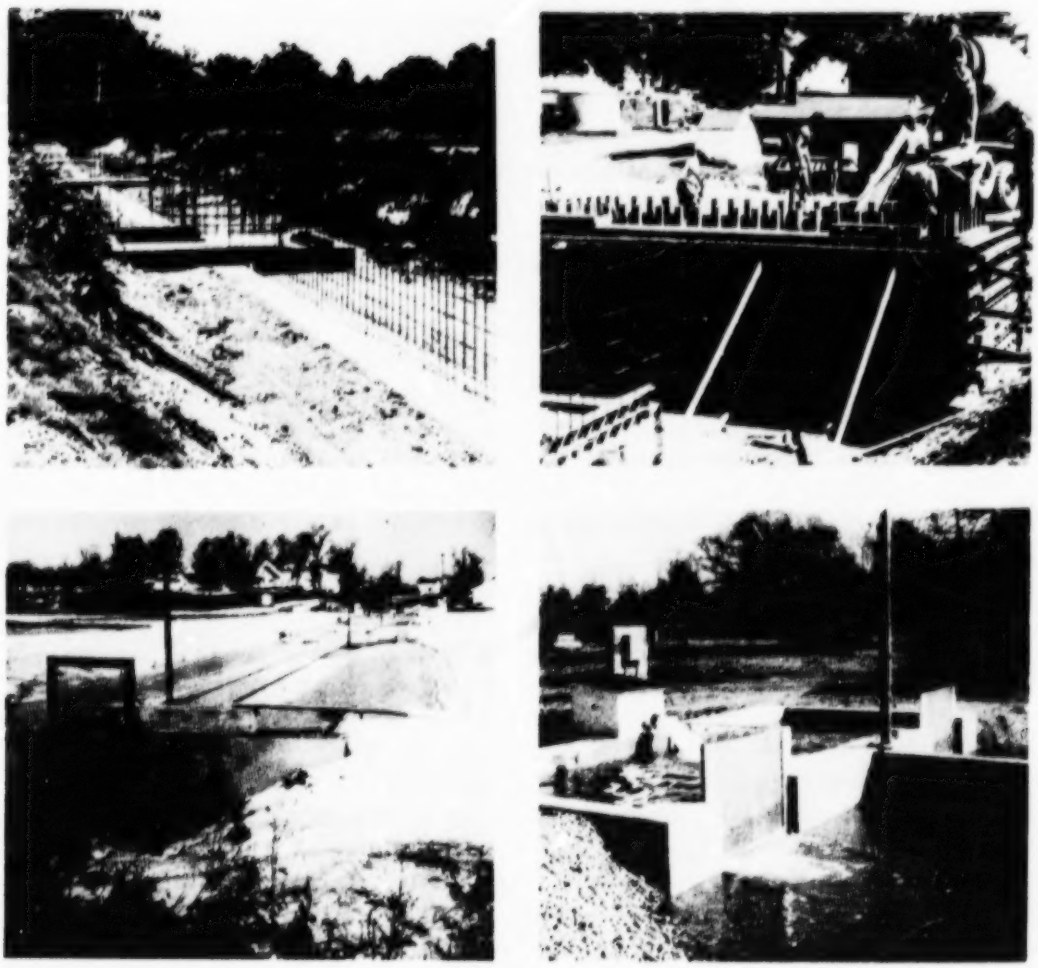

Figure 13

Stages in construction of the experimental streams at Saline:
Upper left--stream bed and footings for dividing wall and mixing boxes.
Upper right--cement forms for water supply box at head of streams.
Lower right--completed water supply box at head of streams.
Lower left--completed facility, from downstream end, during first winter of operation.

SECTION VII

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Dr. M. E. Bender, Virginia Institute of Marine Science, Gloucester Point, Virginia, assisted in planning the project and supervision during early stages of the study.

Dr. R. Hartung, University of Michigan, School of Public Health, Ann Arbor, Michigan, and Dr. W. C. Latta, Institute for Fisheries Research, Michigan Department of Natural Resources, Ann Arbor, Michigan, served as project directors and advisors throughout the study.

Mr. R. C. Barber supervised construction of the experimental streams. The contribution of his entire crew is gratefully acknowledged.

Mr. J. R. Novy assisted with the bioassays and construction of equipment.

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SECTION VIII

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